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THE PASTURE AEDES OF CENTRAL AND NORTHERN CALIFORNIA. THE EGG STAGE: GROSS EMBRYOLOGY AND RESISTANCE TO DESICCATION[1]

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INTRODUCTION

The embryology of insects has attracted the attention of many biologists. In spite of the need for such research with the culicids, relatively few publications deal with mosquito embryology. With the exception of Gander's (1951) these papers have presented rather indefinite and sometimes questionable information. Gander, however, working with *Aedes aegypti* (Linn.), did not record development subsequent to the first segmentation of the embryo. He stated only that it was comparable to that of the mourning gnat, *Sciara*.

The present work is one phase of bionomic research on the pasture *Aedes* of central and northern California. This group of mosquitoes is composed of four species. *A. dorsalis* (Meigen) and *A. squamiger* (Coquillett) inhabit the intertidal salt-grass pastures of the coast. *A. nigromaculis* (Ludlow) and *A. melanimon* Dyar occupy inland irrigated pastures.

Embryological investigations were initially undertaken to establish developmental rates and to point out species differences. Further, it was hoped that additional information would be gained concerning egg diapause suspected in these species.

MATERIALS AND METHODS

Eggs were obtained from field-collected females which were isolated in 15 mm. by 125 mm. test tubes. Each tube was furnished with a moistened strip of absorbent paper for oviposition. The mosquitoes were fed from cotton test tube stoppers saturated with a 10% solution of sugar in defibrinated beef blood. The blood soaked stoppers were replaced with clean ones after feeding had been accomplished. Immediately following oviposition the eggs were placed at selected temperatures. Samples of developing eggs were periodically taken for all species except *A. melanimon*. This mosquito would not oviposit successfully in the laboratory.

Because the original purpose of this investigation was to make gross embryological observations, whole mounts were used. The egg samples were killed and temporarily stored in small shell vials of 10% formalin (DeCoursey and Webster, 1952). In general, the quality of fixation in 10% formalin was only fair. In whole mounts of embryos, intracellular detail was very poor when a nuclear stain was used. In most cases the yolk was rendered opaque. Development was obscured prior to, and in many cases during, the migration of the cleavage nuclei. In addition, this material did not sufficiently harden the embryos; they were easily injured during preparation and mounting. Ten percent formalin was selected because it was the only fixative tested which would not cause the eggs to collapse. It also had the advantage of being a storage medium.

The following are results obtained with other fixatives that were tested. Bouin's (Nelson, 1915) collapsed the eggs, even when they were punctured, and caused extreme distortion of the embryo. Heating the fixative prevented collapse only until the medium had cooled. Dilutions of 20% Bouin's or less prevented collapse but caused greater tissue maceration than formalin. Carnoy's fixative reacted in a manner similar to Bouin's. Fifty to 95 percent alcohol caused the eggs to collapse; lower concentrations resulted in excessive maceration. Fixing in a hot water bath also caused excessive maceration.

The immediate problem in preparing eggs for mounting was to clear or remove the black chorion. Aqua regia (DeCoursey and Webster, 1952) did not completely clear the chorion in spite of repeated renewals of acid and long periods of immersion. Although embryos could be distinguished through the amber shell, structural details were masked at high magnifications.

In order to remove the chorion, various dilutions of sodium hypochlorite were tested (Mor-

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tenson, 1950, and Franco, 1953). A 0.5% solution was chosen for the standard technique. This material would dechorionate all eggs within three hours while an immersion of only thirty minutes was required for young eggs. To avoid injury, early stage embryos were washed in distilled water immediately after the chorion had dissolved. A 1.5% solution dechorionated older eggs more rapidly without causing injury. Stronger solutions were too corrosive. Chorion removal was facilitated after the appearance and thickening of the vitelline membrane around more fully developed embryos. This structure was comparatively resistant to corrosion by sodium hypochlorite. In eggs up to 24 hours old, the vitelline membrane was absent or weakly developed. Because the chorion dissolved unevenly on most eggs, caution was observed with the early stages. When the shell was ready to break through in thin areas, the eggs were placed in distilled water. The chorion was then removed manually with tiny insect mounting pins.

Mayer's carmalum (Nelson, 1915, and Gatenby and Beams, 1950) proved to be very good for staining whole embryos. Overstaining presented no difficulty since the desired intensity could be obtained by destaining with acid alcohol under close observation.

Canada balsam mounting medium gave good results. Piccolite and euparal were eliminated because they distorted the tissue. Although Berlese's fluid caused little distortion, it continued to destain the embryos. It was also considered inferior because it was subject to more rapid deterioration.

Two Coor's porcelain color plates, each with thirty depressions, were used in preparing slide mounts. In this way, as many as twelve egg samples could be handled at one time. Camel's hair brushes were used most successfully for manipulating the eggs. Glass or wire loops and pipettes were too clumsy even though they were less affected by the corrosive preparatory solutions.

The technique adapted for preparing and mounting whole mosquito embryos is as follows:

1. Kill and fix (and store) in 10% formalin, 5 days or longer.
2. Wash in distilled water, up to 5 minutes.
3. Dechorionate in 0.5% to 1.5% sodium hypochlorite, one half to 3 hours.
4. Wash twice in distilled water, up to 5 minutes for each wash.
5. Puncture the vitelline membrane (when present) with a sharpened needle.
6. Stain in Mayer's carmalum, 2 hours.
7. Wash twice in distilled water, up to 5 minutes for each wash.
8. Destain in acid alcohol, 15 minutes or until desired degree of destaining is obtained.
9. Wash in distilled water, up to 5 minutes.
10. Pass through progrssive increases of cellosolve in water. 1:2, 2:1, pure cellosolve, 5 minutes for each change.
11. Mount in Canada balsam.
12. Store mounts on a level surface until the balsam is hard enough for slide use.

The principal disadvantages of this technique were the necessity for using an inferior fixative, the difficulty in clearing or removing the pigmented chorion and excessive handling of the delicate embryos before mounting. Nearly 400 slides were made. Approximately 65% were useful of which about 25% were considered good to excellent.

GENERAL OBSERVATIONS

The slide material showed no major differences in the manner of embryonic development of the pasture *Aedes* at 70, 80, and 90°F. *A. squamiger*, however, was unable to survive at 90°F Additional checks at 60°F. demonstrated that development continued uninterrupted (except *A. squamiger* at 90°F.) at temperatures from 60°F. to 90°F. All egg samples presumed to be in diapause contained mature embryos. Hence, the fully developed larva in the egg undergoes the apparent summer or winter diapause of these mosquitoes. Table I shows the relative rates of development for *A. dorsalis*, *A. nigromaculis* and *A. squamiger* embryos at 70°F., 80°F. and 90°F.

TABLE I

RELATIVE DEVELOPMENTAL RATES (HOURS) OF PASTURE *Aedes* EMBRYOS

TEMPERATURE °F.	*dorsalis*	*nigromaculis*	*squamiger*
90	45–50	45–50	
80	68–74	68–74	216–240
70	90–95	90–95	456–480

It is interesting though not unusual that *A. dorsalis* and *A. nigromaculis* had equivalent developmental rates. The longer time required for *A. squamiger* embryos to mature gives additional evidence that this species is innately slower in all stages. Other data, not reported here, suggest that the embryological growth of *A. melanimon* is similar to that of *A. dorsalis* and *A. nigromaculis*.

EMBRYOLOGY OF *Aedes dorsalis* AT 80°F

Initial inspection of the slides revealed an unexpected amount of detail. Consequently, a sufficient number of mounts was made to offer a clearer and more complete record of mosquito embryology. No apparent gross morphological differences between the species were detected. The embryology of *A. dorsalis* is presented as characteristic of the pattern followed by all three

species. This record was obtained from egg samples taken every one to two hours at 80°F.

A. dorsalis eggs are fusiform, measuring approximately 170 by 530 microns. The micropyle lies in the anterior tip and may be seen in the cleared chorion as a slightly darkened plug (fig. 1). An unfertilized or early cleavage stage egg is filled with finely globular yolk surrounded by a thin layer of periplasm (cytoplasm) which becomes thicker at the anterior and posterior poles. Vacant spaces are formed at the poles by a separation of periplasm from the chorion. Frequently, two or three ligament-like strands traverse these spaces between the terminal periplasm and the chorion.

Because of the opacity of the yolk, early development was obscured in eggs less than 2 hours old. Between the second and third hour the cleavage nuclei begin to migrate to the periphery of the yolk (fig. 2). The first germ cells then bud out into the periplasm at the posterior pole.

At the fifth hour, the cleavage nuclei completely occupy the periplasm. The germ cells at the posterior pole have increased in number to form a pyramid of larger round cells (fig. 3). From the fifth to the twelfth hour development is represented by progressive changes in the blastula. The blastoderm becomes distinct as cell membranes appear around the cleavage nuclei in the periplasm. The cells of the blastoderm progressively thicken until they are cuboidal. As this occurs, the germ cells sink back into the yolk and become covered by the blastoderm. While these changes take place, cells which are evidently vitellophages can be seen throughout the yolk. Between the eleventh and twelfth hour the blastoderm cells elongate on the ventral surface of the yolk. Their columnar appearance is the first indication of germ band formation.

Between the twelfth and the sixteenth hour the blastoderm undergoes a general breakdown which is accompanied by the development of the germ band. The thickened cells on the ventral side of the yolk form a bilaterally ridged band. Its ridges expand somewhat cephalad and caudad to expose a thinner layer of cells lying medially between them. Du Bois (1932), who had seen this median layer in embryos of *Sciara*, designated it as entoderm. She stated that the two ectodermal invaginations, which later appear where the entoderm was formed, push these entodermal cells inward.

The definitive germ band traverses the length of the egg ventrally. Its caudal portion extends over about one-third of the dorsal surface of the yolk posteriorly. Its cephalic extention covers only one-half that distance on the dorsal yolk surface anteriorly (fig. 4).

Within the next 4 hours segmentation of the germ band occurs so rapidly that its origin (cephalad or caudad, etc.) is difficult to establish (fig. 5). The five pregnathal segments are followed by fifteen body segments. The terminal segment, or telson, carries the proctodaeum. The cephalic portion of the embryo extends only a little farther over the yolk, but the caudal portion continues to grow until it almost meets the head. The pregnathal segments give rise laterally to cephalic processes. They are later incorporated into the definitive head and constitute part of the central nervous system. The cephalic processes, at least apically, appear to be trilobed. The germ cells, obscure since the formation of the germ band, are now within the telson. Further development of the germ cells into rudimentary gonads is hidden by other structures. At this stage the embryo and yolk are surrounded by the external embryonic membrane (serosa). Portions of the internal embryonic membrane (amnion), which surrounds only the embryo, are also seen.

Prior to segmentation and outgrowth of the cephalic lobes, the vitelline membrane is not apparent. Soon after this stage the membrane is first seen as a very delicate transparent sack. It closely surrounds the serosa, separating it from the chorion. By the fourteenth hour it becomes a much thicker, harder structure and remains so until hatching.

The position of developing structures, to this point, has been described in relation to the egg. It is more convenient now to describe further development in relation to the embryo itself. In other words, structures described as being dorsal on the embryo will actually be ventral in the egg.

During the following 25 hours, changes are less spectacular and growth proceeds at a steadier rate. At about the twenty-fourth hour, the mouth parts appear as lateral appendages of the sixth, seventh and eighth segments. They grow around the yolk and reach the venter of the embryo at the thirty-sixth hour. At the same time, the brain segments undergo convolution. They fold back upon one another so that their original relative positions become obscure. Nervous tissue destined to become the subesophageal ganglion is pushed ventrally by the growth of the gnathal appendages. The progressive development of the brain from the twentieth to the fiftieth hour is illustrated diagrammatically in figures 12, 13, 14 and 15. During this interval the cephalic region has established its final position in the egg but the caudal region gradually moves back and away from the embryonic head.

By the forty-fifth to the fiftieth hour the embryo has a decidedly different appearance (fig. 6). The embryonic head is divided into two distinct regions, the pregnathal and gnathal areas. The development of the pregnathal region, including the central nervous system, proceeds rapidly. Subsequent to this time it becomes very difficult to follow the changes in the original segments. The gnathal segments begin to enclose the mouth

parts laterally and ventrally. The dorsum of the mandibular segment is uniquely represented by two prominent bulbs of tissue. The thorax and abdomen are still a series of undifferentiated metameres.

At about 38 to 40 hours the stomodeal and proctodeal invaginations begin to grow rapidly, forming the primative foregut and hindgut. At the apex of each of these invaginations, nebulous groups of darker staining cells—the entoderm rudiments—are carried inward. At this stage the eye spots are evident in dechorionated eggs; however, their outlines were no longer sharp after staining and mounting.

When the egg reaches an age of 60 to 65 hours, further gross changes are seen (fig. 7). The embryo has shortened so that it lies entirely on the vertral side of the yolk. The cephalic region is very distinctive, displaying additional pregnathal development. The maxillae and labium are completely surrounded by the body wall. The definitive buccal cavity is evident between the maxillary segment and the pregnathal head region. It appears that the mandibles are not surrounded by the body wall. They evidently drop into the buccal cavity anterior to the funnel shaped esophagus. The progressive development of the mouth parts to this stage is shown in figures 9, 10 and 11.

The thoracic segments are, by this time, longer and broader than the abdominal segments. Because the telson has almost completely disappeared as a result of the proctodeal invagination, the abdomen consists of 9 segments at this stage. The eighth segment gives rise to a bilaterally forked appendage which later forms the siphon (fig. 7a). The lateral extensions of the seventh, eighth and ninth abdominal segments and the thoracic segments have grown a little more than half way around the yolk mass.

Soon after the fiftieth hour, the stomodeal and proctodeal invaginations have reached their limits. The entoderm rudiments have been carried inward as a result of this growth. Each rudiment sends out a thin strand of tissue dorsolaterally along each side of the yolk. After they join, dorsal growth of the entoderm is rapid. Subsequently, a sheath of entoderm separates the rest of the embryo from the yolk. The ventral growth of the entoderm keeps pace with that of the body wall.

Growth and differentiation are rapid from the sixtieth to about the seventy-second hour when the embryo reaches maturity. The main body regions become very distinct. The egg-burster is clearly visible dorsally between the eyes. The mouth parts acquire their final forms. The thoracic and abdominal portions of the body wall, followed by the entodermal midgut, rapidly surround the remainder of the yolk. Soon after this occurs the siphon assumes its definitive form by the fusion of its lobes. Finally, the larval cuticle with its distinct pattern of hairs, tufts and setae becomes evident (fig. 8).

During these final stages the serosa and amnion remnants disappear, evidently being resorbed into the body cavity. The larva is then surrounded only by the tough, transparent, vitelline membrane. Upon hatching, the vitelline membrane and chorion crack in the cervical region. There seems to be a definite weakening in this area, forming a line of dehiscence. It is not discernible prior to hatching.

Gander (1951) observed a rotary movement of the larval head following the completion of embryonic development. He believed that this movement, aided by the egg-burster, created a preformed breaking point in the chorion. This activity alone was not sufficient to burst through the chorion, but at the time of hatching, the *A. aegypti* larva stretched out longitudinally causing the final rupture. Gander stated that the rotary movement of the head might take place weeks or months before actual hatching. This rotation of the head was not seen in the eggs of the pasture *Aedes*; however, no other factual explanation of the uniform dehiscence of these eggs can be offered here.

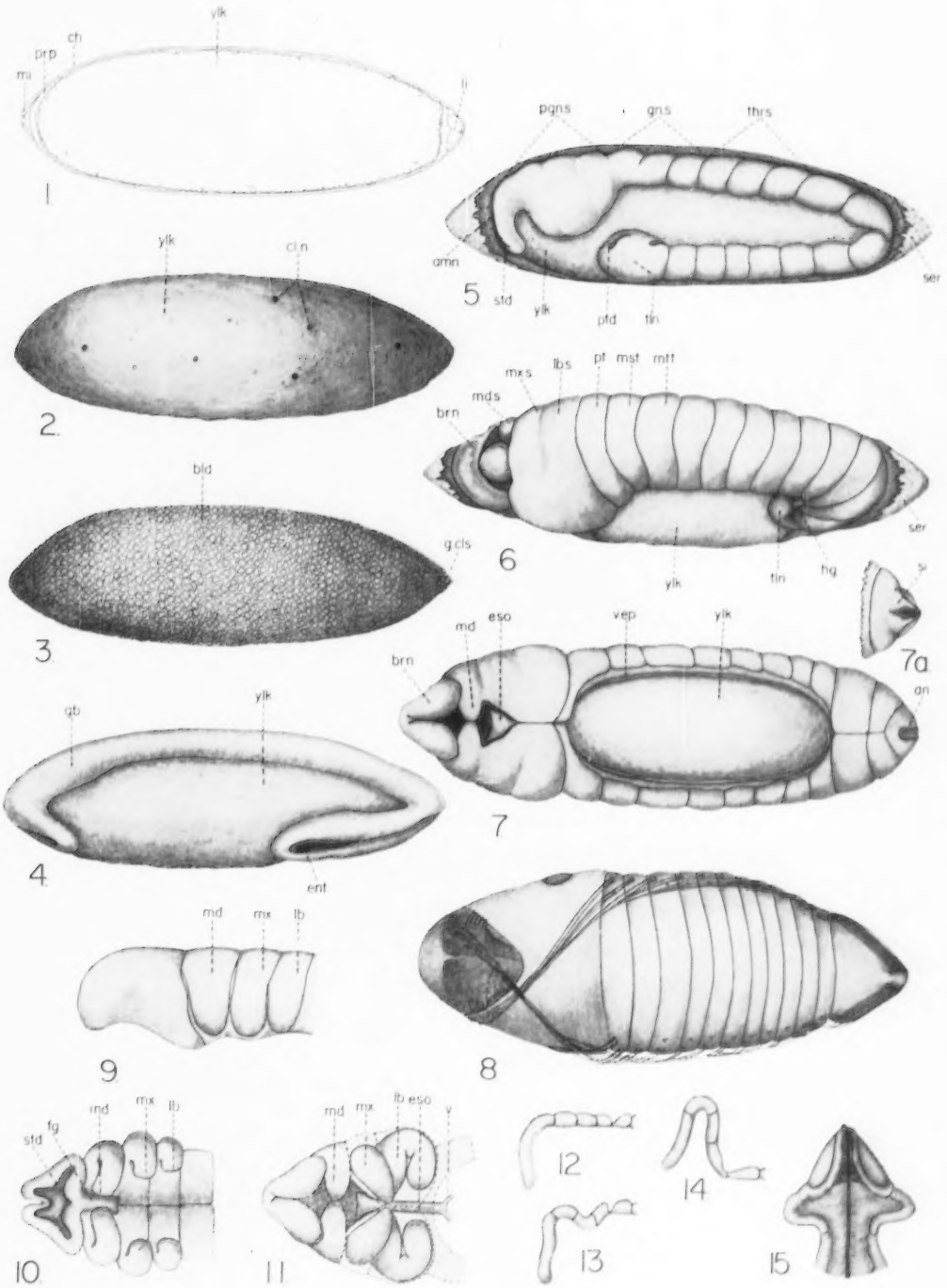
An outstanding characteristic of this embryological picture is that the embryo does not involute at any time. In addition, no part of it was observed distinctly to sink into the yolk mass as in *Sciara* (Du Bois, 1932). Movements are restricted to extension and contraction over the yolk surface. Bodenstein (*in* Roeder 1955) stated that, generally, eggs with long germ bands and abundant cytoplasm characteristically

EXPLANATION OF PLATE

Embryology of *Aedes dorsalis* at 80° F. FIG. 1. Unfertilized egg, longitudinal section. FIG. 2. 2–4 hours, lateral view. FIG. 3. 6–8 hours, lateral view. FIG. 4. 12–14 hours, ventro-lateral view. Fig. 5. 16–20 hours, lateral view. FIG. 6. 45–50 hours, dorso-lateral view. FIG. 7. 60–65 hours, ventral view. FIG. 7a. Dorsal aspect of 8th abdominal segment at 60–65 hours. FIG. 8. 74 hours, ventral view, fully developed embryo. FIG. 9. Gnathal segments, 25–30 hours, lateral view. FIG. 10. Gnathal segments, 35–40 hours ventral view. FIG. 11. Gnathal segments, 60–65, hours, ventral view. FIG. 12. Pregnathal segments, 15–20 hours, lateral diagram. FIG. 13. Pregnathal segments, 25–30 hours, lateral diagram. FIG. 14. Pregnathal segments, 35–40 hours, lateral diagram. FIG. 15. Pregnathal segments, 45–52 hours, ventral view.

ABBREVIATIONS

amn, amnion; an, anus; bld, blastoderm; brn, brain; ch, chorion; cl.n, cleavage nuclei; ent, entoderm; eso, esophagus; fg, foregut; gb, germ band; g.cls, germ cells; gn.s, gnathal segments; hg, hind gut; lb, labium; lb.s, labial segment; li, ligament; md, mandible; md.s, mandibular segment; mi, micropyle; mst, mesothorax; mtt, metathorax; mx, maxilla; mx.s, maxillary segment; pgn.s, pregnathal segments; prp, periplasm; pt, prothorax; ptd, proctodaeal invagination; ser, serosa; si, siphon; std, stomodaeal invagination; thr.s, thoracic segments; tln, telson; v, ventriculus; v.ep, epithelium of the ventriculus; ylk, yolk.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display little or limited movement of embryonic material during development.

No thoracic appendages are developed during embryonic life, nor are evanescent rudiments of such structures ever seen to arise. Evidently this apodal development is common in the Diptera. Nevertheless, most insect embryos do develop these appendages as well as others such as the first and second antennae. These appendages usually appear temporarily even though they have long been discarded in postnatal development during the evolution of many species. It is believed that the *Aedes* embryos possessed at least rudimentary first antennae but positive identification of these structures was not possible in the whole mounts.

The embryological development of the pasture *Aedes*, in general, parallels that known for other dipterous insects.

DESICCATION

Ovipositing females of the pasture *Aedes* are restricted to moist soil. Nevertheless, eggs remain viable and produce larvae a year or more after the earth has become dry. From these observations a question arises: If their eggs are resistant to desiccation, why do these mosquitoes oviposit only on moist ground?

A damp oviposition site may be considered to have at least two advantages. First, an area flooded once is likely to be reflooded before dry areas nearby. Therefore, dampness may indicate the possibility of a future medium for larval development. Second, *newly laid eggs* will survive only in a very humid environment.

Gander (1951), working with *Aedes aegypti*, explained that the character of the egg shell changed with age. Chorion transformation was said to render the eggs resistant to desiccation. This was correlated in some way with the color change of newly laid eggs from white to black.

The following work with *A. dorsalis*, I believe, further clarifies the means by which *Aedes* eggs become resistant to desiccation. In the laboratory, unfertilized eggs appear to react normally when maintained at a high relative humidity. The chorion turns from white to black in the customary manner about an hour after oviposition. These eggs also remain turgid for a week or longer in the presence of adequate moisture. Unlike eggs containing fully developed embryos, unfertilized eggs of an equivalent age quickly collapse when exposed to the humidity of the laboratory. Consequently, it is believed that physically induced changes in the aging shell do not render mature eggs resistant to desiccation. The chorion is a non-living, extra-embryonic structure. Therefore, it also seems doubtful that it would change with the growth of the embryo.

Assuming that the chorion is not the major factor, it might be suspected that resistance to desiccation is correlated with embryological development. *A. dorsalis* eggs developing at 80°F. were exposed in the laboratory on a dish about a foot from a 60-watt globe. All eggs up to 20 hours in age collapsed within 5 minutes. Eggs 20 to 27 hours old showed a rapid increase in resistance to desiccation. Twenty-seven hour eggs remained turgid for a day and a half. They were then checked, found to be viable, and discarded. The ability to resist desiccation, in this case, was acquired within a short period—a little more than a day after deposition. The acquisition of resistance was not gradual. It was then suspected that an embryological event or events which occurred during a relatively short period could have been responsible.

At about the twentieth hour at 80°F., major changes occur. The germ band becomes segmented and the embryonic envelopes (amnion and serosa) become apparent. Furthermore, the so-called "vitelline membrane" is first observed as a thin transparent sack. With age this membrane becomes thicker and harder.

It is possible that either the embryonic envelopes or the vitelline membrane, or both, could regulate water loss from the egg. However, when development is completed, the embryonic membranes are no longer present. They are evidently resorbed by the embryo. Contrarily, the vitelline membrane persists throughout the period when the egg is most resistant to desiccation, being discarded with the chorion when the larva hatches. Therefore, it is believed that the vitelline membrane must be largely responsible for the resistance of the egg to desiccation.

The activity of the membrane during the preparation of living or preserved embryos for mounting supports this hypothesis. In very young eggs the vitelline membrane is apparently not yet developed. Once the chorion is dissolved, these eggs are very susceptible to the corrosive action of sodium hypochlorite. When they are transferred to distilled water, they begin to swell within a few minutes. The vitelline membrane of older eggs is highly resistant to sodium hypochlorite corrosion and when washed in distilled water, the eggs do not swell because of water uptake. In addition, it is necessary to puncture the vitelline membrane in order to permit stain penetration. Thus, it is demonstrated that in living or preserved eggs certain fluids (including water) do not easily pass through this structure.

Most workers agree that the vitelline membrane is a transparent, non-cellular layer located beneath the chorion. Its origin is uncertain. Snodgrass (1935) stated that the cell wall of the ovum becomes the vitelline membrane of the mature egg. Johannsen and Butt (1941) presented the view of Korschelt, who stated that the vitelline membrane is the hardened surface of the periplasm.

Baement (1949) stated that the fertilized eggs of *Rhodnius* produce a fertilization membrane

immediately before oviposition. This structure covers the entire inner surface of the shell. At first it is very thin and colorless, but by the sixth day it has been supplemented by additional material. Tests showed that it is impregnated with a high melting point wax. The membrane is comparatively resistant to solvents and semipermeable to salt solutions.

The late appearance of the membrane in *Aedes* eggs and the fact that it becomes thicker and harder with age are in agreement with Korschelt's ideas concerning the vitelline membrane. The characteristics of the fertilization membrane of *Rhodnius* also suggest a similarity between that structure and the vitelline membrane of *Aedes* eggs.

SUMMARY

The embryology of *Aedes dorsalis*, *A. nigromaculis* and *A. squamiger* was studied in order to make specific comparisons. Particular attention was given to the relationship between embryonic growth and diapause in the eggs of these mosquitoes. *A. dorsalis* and *A. nigromaculis* developmental rates are equivalent, while *A. squamiger* embryos require a considerably longer time to mature. This further substantiates the innately slower development of *A. squamiger* in all stages.

At temperatures which are not lethal, growth and differentiation continues without interruption. The mature embryo or larva in the egg undergoes the summer or winter diapause suspected in these species.

The embryology of *A. dorsalis* at 80°F. is typical of all three species at sublethal temperatures. These embryos neither involute nor sink into the yolk mass. Movements are restricted to extension and contraction over the yolk surface. No thoracic appendages are developed during embryonic life, nor are evanescent rudiments of such structures seen to arise. However, it is believed that the embryos possess at least the rudimentary first antennae. Positive confirmation of these structures was not possible in the whole mounts.

It was demonstrated that the vitelline membrane became evident at the time the eggs developed resistance to desiccation. Experimental evidence indicates that this structure probably plays a major role in rendering *Aedes* eggs resistant to desiccation. The origin of the vitelline membrane is discussed.

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CALIFORNIA WASPS OF THE GENUS OXYBELUS (HYMENOPTERA: SPHECIDAE: CRABRONINAE), by Richard M. Bohart and Evert I. Schlinger. Bull. Calif. Insect Survey, vol. 4, no. 4, pp. 103–134, pls. 9–16, 23 maps. University of California Press. 75 cents.

This paper is a welcome addition to the growing list of bulletins of the California Insect Survey covering the insect fauna of that state. The format is similar to earlier numbers of the series. After a brief introduction, the authors present a key to the 40 species of *Oxybelus* occurring in North America. Following this, the 24 species occurring in California are considered (previously only 9 had been recorded from that state!). Under each species is presented a brief discussion of some of the more important characters, notes on geographic variation, and a listing of California and the more significant out-of-state localities where the species has been taken. The accompanying maps show the specific California localities as well as the total range of the species in North America. The drawings are very well done and consist mostly of figures of the postscutellar squamae and propodeal spine, the two sexes being figured separately.

No synonym is given, the reader being referred to another recent paper by the same authors (Pan-Pac. Ent., Oct. 1956) which gives full synonymy and brief distributional data for all the North American species of *Oxybelus*. These two papers together constitute, in effect, a revision of the genus for the United States, Canada, and northern Mexico. Such a revision has been much needed, since the group has scarcely been touched in a critical manner since Robertson's 1889 "Synopsis". Although these small wasps are not often encountered by the average entomologist, some of the species are common enough in areas where there are suitable nesting sites. Their prey consists of a variety of Diptera, including many economically important species. In some species the prey is carried to the nest impaled upon the sting, though how it is placed on the sting and made to stay there is unknown. It is to be hoped that these excellent papers of Bohart and Schlinger will stimulate much-needed biological studies of these interesting wasps.—Howard E. Evans.

BAT TICKS OF THE GENUS ARGAS (IXODOIDEA, ARGASIDAE)

2. SECRETARGAS NEW SUBGENUS AND A. TRANSGARIEPINUS WHITE, 1846, ITS ADULT AND IMMATURE STAGES; WITH A DEFINITION OF THE SUBGENUS ARGAS[1]

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INTRODUCTION

Argas transgariepinus was briefly described by White (1846) from three cotype specimens, now in British Museum (Natural History), found somewhere in South Africa (i.e. "across the Gariep River", the old name for the Orange River). Neumann (1901) reported a specimen from Cafrérie (Eastern Province, Union of South Africa) under the probably synonymus name *A. kochi* (Paris Museum). Berlese (1913) commented on two Italian specimens, one on the wall of a building and the other biting a child. In Egypt, this is a rare and highly secretive tick but over a period of years more than five hundred specimens have been collected from local populations (Hoogstraal 1952, 1954) and numerous others have been reared in the laboratory. A single specimen from Teruel, Spain, was seen by Nuttall (Hoogstraal 1954). Recently I have been privileged to examine two larvae of this species from *Pipistrellus savii savii* (Bonaparte, 1837) from Syracuse, Sicily; these are part of a lot (T23) collected in July, 1955.

Bedford (1934) considered the single known specimen of *A. kochi* Neumann, 1901, to be distinct from *A. transgariepinus* because of supposed differences in prominence of tarsal humps, an inconclusive factor considering possible individual variation and paucity of material.

A. transgariepinus has not been described from fresh specimens since 1846, except for a brief diagnosis of Egyptian adults (Hoogstraal 1952) together with comments on inaccuracies in previous illustrations of the type specimens.

The present full description and illustrations of both sexes and of the immature stages of this species is part of an effort to establish a practical concept of species relations and subgeneric criteria in the family Argasidae. Previous proposals to subdivide the genus *Argas* into several independent genera obscure the obvious natural relationships among members of this group. The genera of Ixodoidea have, in general, become well established following the monumental work of Nuttall and Warburton (1908 to 1924), species in the order are few, and individual genera are quite homogeneous. Little "practical" or academic utility can be gained from tampering with present concepts of most tick genera. Phylogenetic trends among ticks, especially in the genus *Argas*, are lucidly demonstrated on a subgeneric level.

Secretargas new subgenus

Parasites of which bats (and possibly lizards) are hosts of predilection. Body flat, outline quadrate to subcircular, width equalling at least two-thirds of length. Sutural line (lateral suture) present; hood and ventral "paired organ" absent. Integument with small, ridge-like granulations and rough mammillae; body margin striated dorsally and ventrally; discs moderately distinct, not arranged in clearly radiating rows. Mouthparts concealed from dorsal view. Legs fairly short and almost entirely concealed from dorsal view; tarsi with subapical dorsal protuberances.

Type of the subgenus: *Argas transgariepinus* White, 1846. Cotype specimens in British Museum (Natural History).

Remarks: A. transgariepinus is the only species presently known in the subgenus. It differs from members of the subgenus *Argas* in body outline and proportions, granular and mammillated integument, shortness of legs, and presence of subapical dorsal protuberances on tarsi. The new subgeneric name, *Secretargas*, proposed here refers to the exceptionally secretive habits of this species; by another definition of the same word (*L. secretus*) it may also be taken to indicate the separateness of this subgenus from the subgenus *Argas*, with which, however, it has many salient characters in common.

Subgenus **Argas** Latreille, 1796, 1802

Parasites of which birds are usually hosts of predilection. Body flat, considerably longer than wide, width distinctly less than two-thirds of length; sutural line (lateral suture) present; hood and ventral "paired organs" absent; body margin dorsally and ventrally with striae or "cells". Integument leathery with minute folds and small, smooth mammillae; discs arranged in distinctly radiating rows. Mouthparts concealed from dorsal view. Legs moderately long, much longer than body width, tarsi lacking dorsal protuberances.

Type of the subgenus: Acarus reflexus Fabricius,

[1]Research Report NM 005 050.39.47. The opinions and assertions contained herein are the private ones of the author and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large. Accepted for publication Jan. 16, 1957.

1794, Latreille, 1796, 1802. Types not known to exist.

Other species in subgenus: This subgenus also contains *A. persicus* (Oken, 1818), *A. hermanni* Audouin, 1827, *A. cucumerinus* Neumann, 1901, *A. lagenoplastis* Froggatt, 1906, *A. delicatus* Neumann, 1910, and ?*A. passerinus* Schulze, 1941. Although it appears likely that several species or subspecies closely related to *A. reflexus* parasitize wild birds in various parts of the world, the

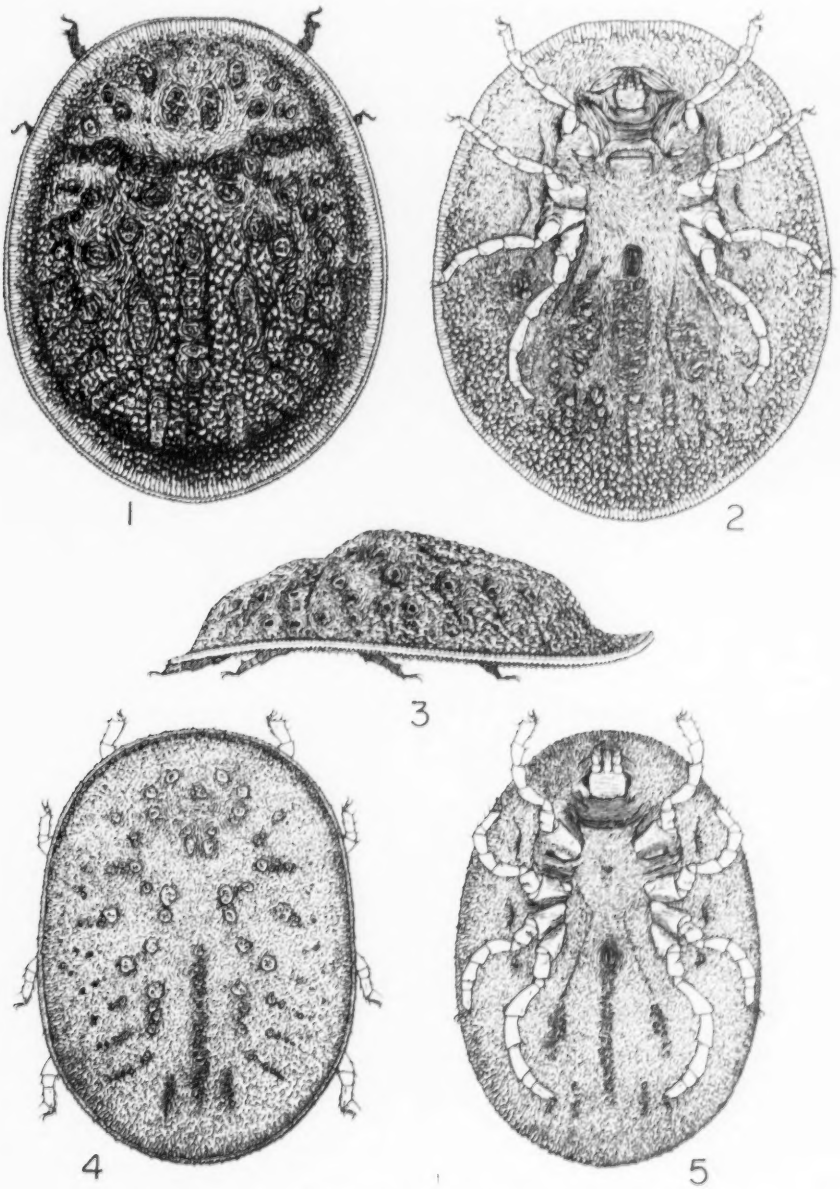

FIG. 1. Female, dorsal view (with partly digested blood meal).
FIG. 2. Female, ventral view (with partly digested blood meal).
FIG. 3. Female, lateral view (with partly digested blood meal).
FIG. 4. Nymph, dorsal view (medium size, unengorged).
FIG. 5. Nymph, ventral view (medium size, unengorged).

status of individual members of this complex is at present difficult to discern.

Remark: The subgenus *Argas* is redefined at this time in order to illustrate the affinities and differences between this subgenus and *Secretargas* (see Remarks above).

Argas (Secretargas) transgariepinus White, 1846

The Secretive Bat-Argas

ORIGINAL DESCRIPTION (page 318): "*Argas transgariepinus*, White; figured on plate 2, fig. 4. Body ovoid, minutely verrucose, upper surface,

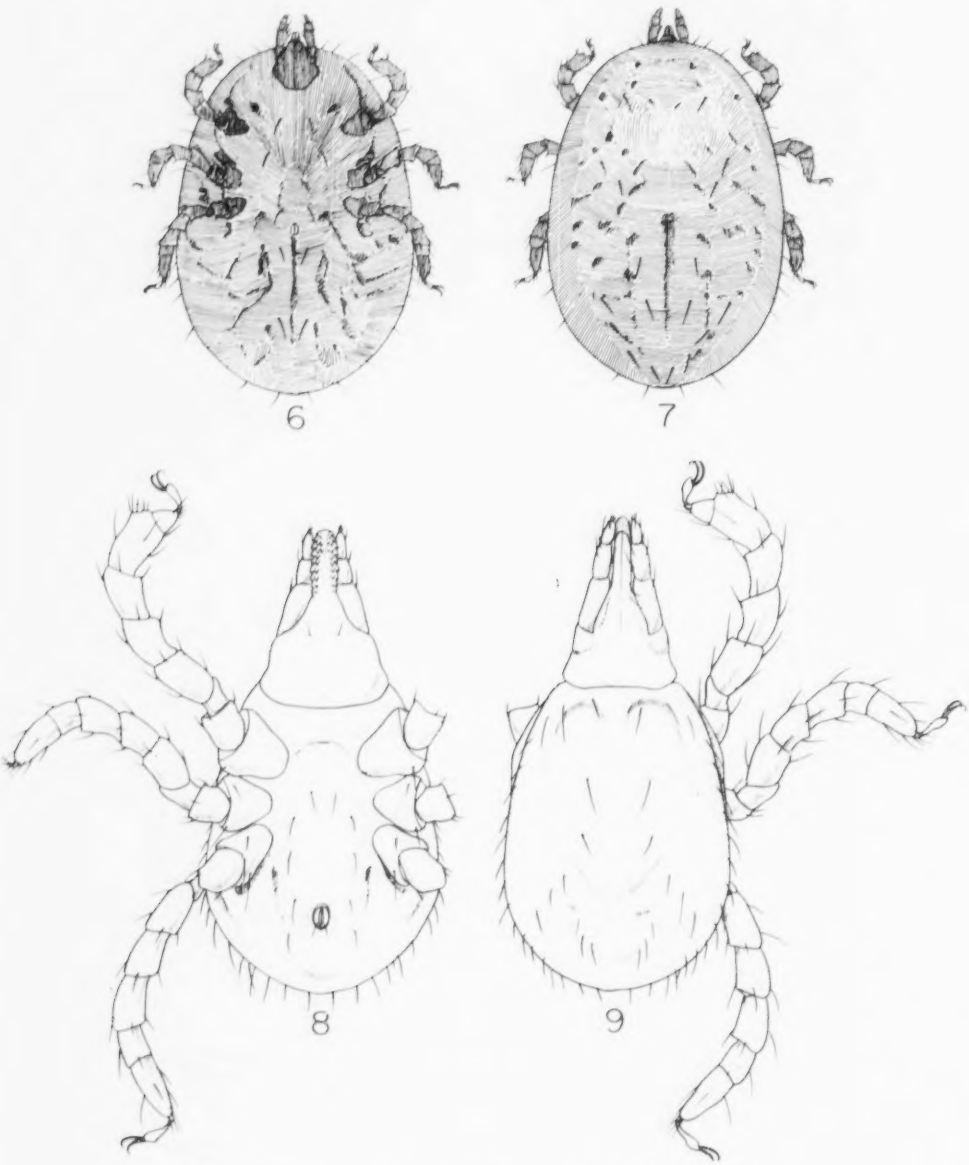

FIG. 6. Larva, ventral view (fully engorged, hypostome broken).
FIG. 7. Larva, dorsal view (fully engorged, hypostome broken).
FIG. 8. Larva, ventral view (unengorged).
FIG. 9. Larva, dorsal view (unengorged).

except on the edges, pitted, margin entire. This *Argas* is of a brownish red; the legs rather paler."

REDESCRIPTION (OF EGYPTIAN POPULATIONS, GIZA PROVINCE) FEMALE: Measures from 8.50 mm. to 11.50 mm. in length and from 6.50 mm. to 9.00 mm. in width; average specimen 10.50 mm. long and 8.75 mm. wide. *Body* (unengorged) thin and flat, concealing mouthparts and most of legs; outline quadrate to subquadrate with more or less bluntly rounded posterior and anterior margins (degree of bluntness somewhat variable; posterior margin frequently more gradually rounded than anterior margin), lateral margins parallel to slightly convex; (engorged) central dorsum elevated leaving a flattened peripheral flange; a narrow, transverse integumental depression across anterior third of body. *Color* (unengorged) lemon to reddish yellow, brownish red, or yellowish brown; (engorged) darker to black centrally with flange lighter or same color as central area, becoming progressively darker as blood is digested.

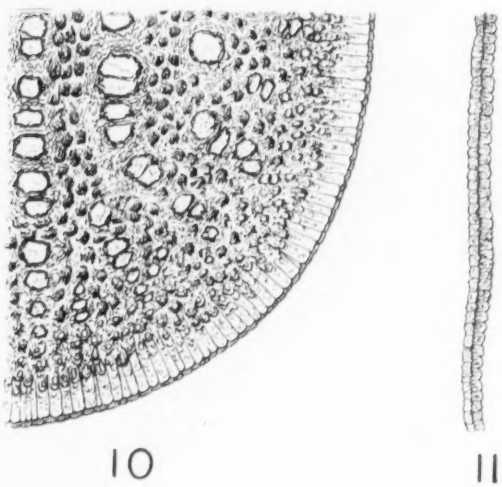

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FIG. 10. Female, details of dorsal integument, lower right quadrant.

FIG. 11. Female, details of lateral integument.

Dorsal integument irregularly granular especially anteriorly, granulations narrow, ridgelike, tending to run in concentric lines around discs; roughly mammillated posteriorly and sublaterally, with minute granulations among mammillae; deeply and irregularly striated laterally; surface transversely depressed across anterior third and frequently with three longitudinal linear depressions on posterior half. *Discs* moderately distinct, not arranged in clearly radiating rows; surface of individual discs slightly depressed from periphery and frequently grooved (discernibility of discs depends largely on integumental coloring and degree of engorgement). *Lateral integument* with striations from dorsal and ventral surfaces interrupted by sutural line (lateral suture) that completely encircles body. *Ventral integument* quite similar to dorsal surface except that mammillae and granulations are finer; discs arranged as illustrated. *Hood* absent. *Hairs* minute and pale, rare except for a single one between each lateral striation both dorsally and ventrally and for a number of irregular rows on the ventral surface.

Capitulum small, in shallow camerostome situated slightly anterior of level of coxae I. *Basis capituli* ventrally rectangular with mildly convex lateral margins and a row of at least three short hairs laterally. *Hypostome* slightly over twice as long as wide; apex bluntly rounded, emarginate, with a weak corona; dentition on apical half posterior of corona 2/2 in three or four files, on basal half 3/3 in four or five files but these denticles much smaller, weaker, and less distinct; with two pairs of posthypostomal hairs, the anterior arising at level of midlength of basal palpal segment and extending to penultimate segment, the posterior arising at level of insertion of basal palpal segment and extending to posterior half of same segment. *Palpi* extending beyond hypostome by full length of segment 4, segments subglobose, progressively smaller from basal to apical segment.

Eye absent. *Spiracular plates* small, situated lateral to coxae IV. *Genital aperture* a transverse slit situated at level of base of coxae I. *Anus* elliptical; anterior half situated at level of apical half of coxae IV; approximately six fine, pale hairs arising from each valve. *Ventral "paired organ"* and *ventral grooves* absent. *Coxal* and *supracoxal folds* present but not distinct. *Coxal organ* aperture visible at base of coxae I.

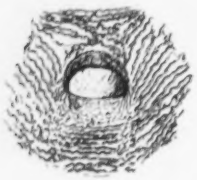
Legs arising from anterior half of body, fairly short and stout; I to III subequal; IV somewhat longer. *Coxae* II to IV transversely narrowly subtriangular, separated from each other by a deep, narrow groove; I more distant and somewhat larger, bounded posteriorly by aperture of coxal organ. *Tarsus* I with a much elevated, two-humped subapical dorsal protuberance, the proximal hump with two pairs of hairs and the distal hump with one long and two short hairs; abruptly tapered distal of hump. Tarsi II to IV each with a single humped, less elevated subapical dorsal protuberance bearing a pair of long hairs; tapering distal of hump less abrupt than that of I, IV even more gradually tapered; each tarsus with two pairs of lateral hairs, three pairs of apical hairs, and three or four pairs of ventral hairs. *Pads* small. *Claws* of I very small and not equalling height of subapical dorsal protuberance; II and III somewhat larger; IV more massive and more strongly recurved.

MALES: Similar to female except for smaller size and difference in genital aperture. *Measures* from 6.50 mm. to 8.75 mm. in length and from 4.75 mm. to 6.75 mm. in width; average specimen 7.50 mm. long and 6.00 wide. *Genital aperture* a small semicircle situated at level of base of

coxae I. *Tarsi* with somewhat smaller subapical dorsal protuberances and with two pairs of dorsolateral hairs that are not observed in female; claws of IV largest but relatively less massive than in female.

NYMPH: Resembling adults except for size although large nymphs may equal all but large females and have a slight groove in place of the adult genital aperture. The integumental granulations and mammillae are finer than in adults and the capitulum is closer to the body margin. Features of the capitulum are quite similar to those of the adults. It is noteworthy that the aperture of the coxal organ is clearly visible; and, except for size, reduction in numbers of a few groups of hairs, and less pronounced tarsal humps, there is little difference between first and later instar nymphs. First instar nymph (unengorged) measures approximately 3.80 mm. long.

LARVA (UNENGORGED): Measures approxi-



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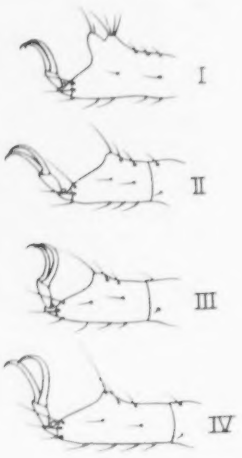

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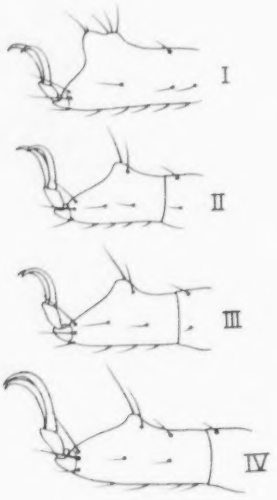

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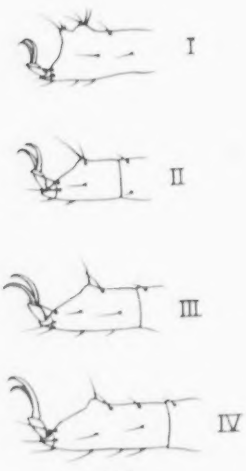

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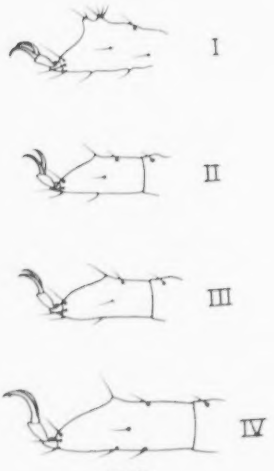

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FIG. 12. Male, genital area.
FIG. 13. Male, capitulum.
FIG. 14. Male, tarsi I to IV.
FIG. 15. Female, capitulum.
FIG. 16. Female, tarsi I to IV.

FIG. 17. Nymph, large, capitulum.
FIG. 18. Nymph, large, tarsi I to IV.
FIG. 19. Nymph, first instar, capitulum.
FIG. 20. Nymph, first instar, tarsi I to IV.

mately 1.20 mm. from apex of hypostome to posterior body margin; color pale white; mouthparts arising from anterior margin of body; lateral margins of body parallel to slightly convex, posterior margin bluntly rounded. *Hairs* dorsally on body integument numbering approximately sixteen pairs laterally, three pairs anteriorly, two pairs centrally, three pairs posteriorly sublaterally, and three pairs posteriorly submedianly; ventrally five submedian pairs extending from anterior level of coxae II to posterior level of anus.

Capitulum comparatively large, situated anterior of body. *Basis capituli* with bluntly rounded base ventrally, slightly concave or straight base dorsally, lateral margins indented ventrally, converging anteriorly and shorter dorsally. *Hypostome* from basal denticle approximately twice as long as greatest width; bluntly rounded anteriorly with very slight indication of weak corona, lateral margins parallel; dentition 2/2, denticles numbering approximately ten in each file; an inner pair of files composed of two to four denticles anteriorly; posthypostomal hairs minute, one pair. *Palpi* extending to apex of hypostome; segment 2 only slightly shorter than combined length of 3 and 4; segment 4 slightly shorter and narrower than 3.

Anus situated at level of trochanter III, with a long hair from each valve.

Legs moderately long and stout. *Coxae* progressively larger from III to I, basally rounded and divergent laterally, partially contiguous. *Tarsi* gradually and slightly tapering, I with slight indication of subapical dorsal protuberance; pads relatively large; claws small.

LARVA (FULLY ENGORGED): Body shape similar to that of nymphs; measures approximately 3.30 mm. long. Basis capituli distorted and concealed from dorsal view; hypostome frequently broken during removal from host. Legs partially visible from dorsal view; coxae separated by integumental stretching. Hairs frequently missing.

BIOLOGY

A. transgariepinis in Giza Province, Egypt, is a highly secretive parasite of bats, chiefly of those solitary species, noted below, that rest singly or in twos or threes in small clefts in cliffsides in the arid, barren desert some miles from vegetation. Seldom found in more humid caves in which gregarious bats roost, it frequently undergoes months of starvation owing to the irregular visits of its hosts, which rest in any of a great number of clefts available in this area. Strenuous pickaxing is necessary to obtain specimens from the narrow cracks and interstices in which these ticks conceal themselves. Only an infinitestimal proportion of wild specimens are found engorged. However, since we have observed nucleated erythrocytes in a few of these engorged individuals, it is apparent that geckos, the only other vertebrates inhabiting these situations, sometimes also serve as hosts.

Larvae in Egypt are commonly found on *Otonycteris h. hemprichi* Peters, 1859, and once on *Rhinopoma harwickei cystops* Thomas, 1903. In Sicily, *Pipistrellus s. savii* (Bonaparte, 1837) serves as a host. As already stated, geckos may also be parasitized by this tick. All stages feed on any species of bat in the laboratory, and adults and nymphs become partially engorged on human beings. Life cycle studies are presently under way and will be reported separately.

Dr. Gertrud Theiler has kindly called my attention to certain facts concerning the locality in which the original specimens of *A. transgariepinus* were collected. Although it has been alleged by several reviewers that these collections were made in Basutoland, Methuen's travels did not take him anywhere near Basutoland. "Transgariep" (i.e. across the Orange River) indicates an area between southeastern Orange Free State and present day eastern Bechuanaland, which is a dry region.

SUMMARY

A. transgariepinus White, 1846, is known only by four specimens from South Africa (one of which is the probably synonymous *A. kochi* Neumann, 1901), two from Italy, two from Sicily, one (now lost) from Spain, and many from Giza Province, Egypt. Egyptian populations of both sexes and of the immature stages are described and illustrated. In Giza Province, Egypt, this tick secretes itself in cracks in clefts of arid, barren desert cliffsides where solitary bats occasionally roost; it feeds on these as well as on geckos. In the laboratory it also feeds on human beings.

The subgenus *Secretargas* is proposed for this species, which has unique characters in common with members of the subgenus *Argas*, which is redefined for comparative purpose.

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A NEW ERA IN IDEAS OF SYSTEMATIC RELATIONSHIPS OF WORLD TABANIDAE (DIPTERA) INAUGURATED BY MACKERRAS, AND ITS IMPACT ON NOMENCLATURE OF HORSEFLIES OF THE WESTERN HEMISPHERE[1]

CORNELIUS B. PHILIP

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The family Tabanidae has always been popular in the Diptera both to systematists and to those interested in haematophagous parasites as potential disseminators of disease agents. It is within three years of a century since Loew (1860) proposed the first major, suprageneric cleavage in the family based on the presence or absence of hind-tibial spurs to provide two subfamilies or (later) sections. The contents of the family, however, have remained well defined with the exception of certain excluded minor elements of which the latest are the Australian and/or North and South American genera, *Pelecorrhynchus*, *Bequaertomyia*, and *Heterostomus*.

Most annoyingly, almost all good systematic characters in the family have shown exceptions, and the character of hind-tibial spurs is no exception (to "prove the rule" as it were). As knowledge of the family was inevitably refined and expanded, the need for new concepts became increasingly evident. A start toward this was made by Lutz (1914) when he established his two Sections, Opistanoplae and Opistacanthae, so that he could provide more subfamilies. This merely had the effect of raising the previous subfamily category by another level and was still based on a dichotomous concept of the presence or absence of the spurs. The arrangement, however, enabled Enderlein (1925) to introduce his elaborate and often artificial system of subfamilies and tribes.

Systematization of many other families of the Diptera has steadily progressed, aided often by critical studies of genitalia of one or both sexes. Limited studies by several workers, however, have depicted what we now come to realize is a false picture of the complete sterility of this field in the Tabanidae. Townsend (1935, 1942) ably discussed the growing opinions of the fallibility and limitations of dependence on "external fly characters only." The time was ripe for I. M. Mackerras, Director of the Queensland Institute of Medical Research in Australia, to try his microscalpel on the genitalia of the world species, and not, as heretofore, on just a limited fauna.

The results (Mackerras 1954, 1955a, 1955b) were surprising and fruitful, at least as far as he has progressed, chiefly down to the tribal level. This is hardly an accident since his interest stems from long attention to the tabanid fauna of the Australian region, which, together with related elements in the Neotropical fauna, is the oldest and in many ways richest on earth in suprageneric diversification in this modern insect family. Elements of the family in this and other areas well illustrate Townsend's (*op. cit.*) ideas of "embryonic, nascent, mature, and senile" groups in point of evolutionary development, and Banks' (1910) comments on dynamic insect distribution. Mackerras (1955a, p. 455) recognized this in his discussion of "several different sorts of genera" of Tabanidae.

Many genera among the more primitive pangoniines are common, as well as peculiar, to southern and western South America and Australia, with relatives in South Africa. Conversely, an even more biologically successful development in point of speciation has taken place in the Northern Hemisphere in the genera *Chrysops*, *Chrysozona* (=*Haematopota*), and *Tabanus sens. lat.* with radiations southward (*e.g.*, *Chrysozona* in Africa). It is the purpose of the present discussion to call attention to the revised conception of systematic relationships in the New World only, although Part IV on Tabaninae, of major moment to the Nearctic fauna, is still in preparation by Mackerras. He has already discussed evolutionary and zoogeographic trends, two major features of which are an ancient, southern or "Gondwananaland" pathway and a more recent specialization and radiation in the Northern Hemisphere. The richness of primitive derivatives in South America has suggested to Oldroyd[2] the further hypothesis that the family had its origin in tropical South America and radiated in two main branches both southward and northward. Good evidence supports the views of these two authors. Mackerras' revision, which provides four basic subfamilies in the Tabanidae (illustrated in Fig. 1), appears acceptable. The heads and fronts of generalized representatives are shown in Fig. 2.

THE CHARACTERS

As is further emphasized in Mackerras' studies, previous reliance on single sets of external

[1]Accepted for publication Jan. 16, 1957. Read at the Fourth Annual Meeting of the Entomological Society of America, New York, N. Y., December 19, 1956.

[2]While this paper was in preparation, Mr. Oldroyd generously forwarded proof pages of his pertinent discussion in Horseflies of the Ethiopian Region, Vol. III, in press.

characters in the different subfamilies has led to misplaced emphasis and erroneous association of genera, a fact further emphasized in Oldroyd's studies of the Ethiopian fauna (*e.g.*, the hairy-eyed group of Tabanini, 1954, p. 133). Fortunately, Mackerras has not relied on genitalic characters alone, but he has used them in complement with time-proven external characters to produce a more natural rearrangement of related groups which makes quickly apparent why these new concepts have resulted in major and epochal advancement, as well as in realization of the artificiality of a number of previous groupings. Exceptions to generalized external characters as guides have also now come into better perspective, and explanation of parallel or converging evolutionary development is more readily acceptable. Tribes and subfamilies have finally passed the stage of mere mechanical, systematic devises, and have become comprehensible, phylogentic labels with better zoogeographic significance.

It is still painfully evident that a given character that is reliable in one subfamily for generic differentiation is not necessarily of equivalent stability in another. The closure of wing cell R_5 in most, not all, pangoniine genera is a reliable, differential generic character, whereas, in many Tabaninae it is not even of specific reliability, for here the condition varies occasionally even in the two wings of the same individual, or in the two sexes of the same species (*e.g.*, *T. petiolatus* Hine ♀ with cell closed and syn. *T. yulenus* Phil. ♂, with cell open as confirmed by rearings of C. M. Jones); yet Enderlein (1925) has used this character to include a conglomeration of species in his artificial subfamily Bellardiinae based solely on closure of this cell.

The non-setulose condition of the basicostas (subepaulets) at the base of the wings is considered a primitive characteristic. In many of the more specialized Tabaninae, this structure has become setulose and this furnishes a broad differential character in this subfamily, but Mackerras and Oldroyd have further implied that there is a reversion to the primitive, bare condition in a few specialized species (*e.g.*, *Tabanus apricus* Mg. of Europe). Unfortunately, apparent exceptions again have occurred in some obvious South

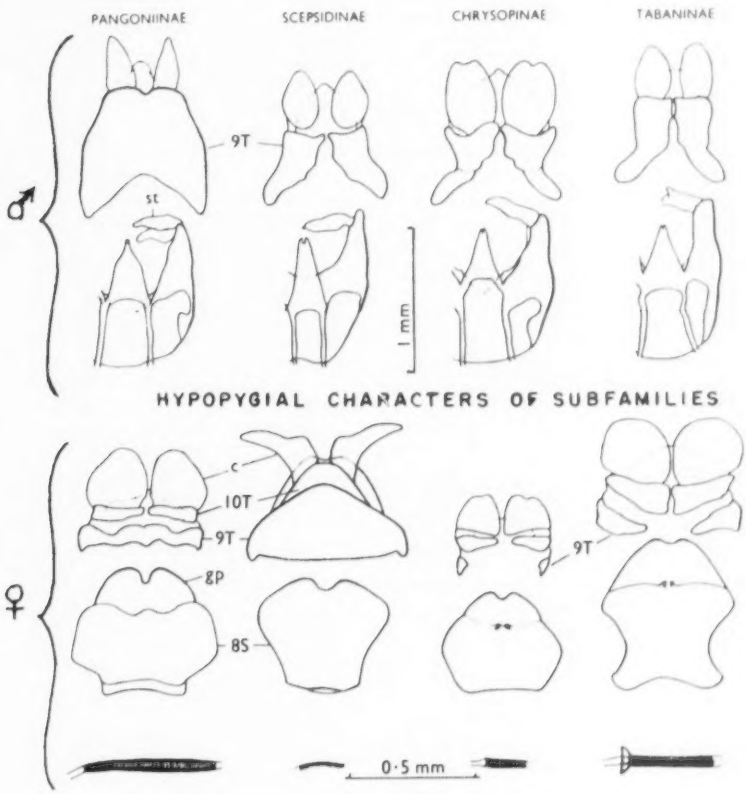

FIG. 1.—Hypopygial characters of subfamilies (adapted with permission from Mackerras, 1954. 8S, eighth sternite; 9T, ninth tergite; c, cerci; gp, anterior gonopophyses; st, style).

American diachlorine relatives of *Stenotabanus* in which this structure is sparsely setulose, probably an example of convergence with the specialized Tabanini. This weakens the arbitrary differentiation, for key purposes, of the Tribes Diachlorini and Tabanini but in my estimation does not invalidate recognition of these two as actively developing, phylogenetic elements.

Progressive amalgamation or reduction of segments in the antennal flagellum, specialization of the basal plate frequently with basal hook or thumb, processes on the annuli, and disappearance of the ocelli are useful supplemental characters that require caution in their interpretation. Parallel progression probably accounts for extreme reduction of annuli in Haematopotini, *Glaucops*[3] in the Tabanini, and in the chrysopine *Pronopes*, *Merycomyia* and *Dasycompsa*. The strong, antennal hook has obviously appeared more than once in different groups. While the specialized species tend to lose their ocelli, I suspect that the three ocelli (probably nonfunctional) of the Nearctic *Hybomitra cincta* (Fabr.), for which Enderlein (1925) erected the artificial genus *Dasyommia*, represent resurgence rather than degenerative persistence of these structures. Possibly the same is true in American *Leucotabanus*.

The bare or variably hairy condition of the eyes, requiring experience to interpret, has long been in systematic use, bare eyes being considered more primitive. Hairiness, like that of the body, is more specialized and is often accentuated in cooler, drier or higher climates by convergence in all three major subfamilies. This has been a useful character, particularly in conjunction with an ocelligerous tubercle, in attempts to divide the cumbersome genus *Tabanus sens. lat.*, and to exclude elements widely divergent from the bare-eyed genotype, *T. bovinus* Linn. Whether the divergent groups are considered on a generic or only a subgeneric level, again unfortunate intergradation of a few species complicates determination of group limits, and it is equally difficult to decide where to stop "lumping" when one reaches such distinctive groups as *Atylotus* and *Therioplectes*. Nevertheless, the important group set up in the mostly northern *Hybomitra* is a useful taxonomic unit. Analogous difficulties are encountered in other dynamically developing groups, such as the pangoniine *Scaptia* and chrysopine *Mesomyia*. Whether intensified genitalic studies will aid in clarifying any of these generic difficulties remains for the future.

Mackerras' genitalic studies have demonstrated the importance of undivided ninth sternites, plus, in the males, bifid styles, in differentiating the Pangoniinae from the higher groups, and of collars on the spermathecal ducts of females, to set apart the specialized Tabaninae from the Chrysopinae (Fig. 1). These are characters of phylogentic significance, but a few exceptions are noted even in these characters. Ovazza and Taufflieb (1954) had also noted differences in the spermathecal ducts ("manchons") between females of the old Pangoniinae (mostly Chrysopinae) and the Tabaninae but overlooked the undivided ninth tergite in the pangoniine *Dorcolaemus*.

As Mackerras (1954) has pointed out, the information on immature stages of Tabanidae is far too meager for fruitful correlation as yet, but what there is, is consistent with the regrouping in his systematic revision. In his own words, "The groups appear to be natural, in that the genitalic characters rarely tend to merge into one another, there is no evidence of parallel

[3]It is questionable in the writer's opinion if such species as Nearctic *T. daedalus* Stone and Siamese *T. ochros* Sch. Stek., also only triannulate, are congeneric or merely represent convergent specialization.

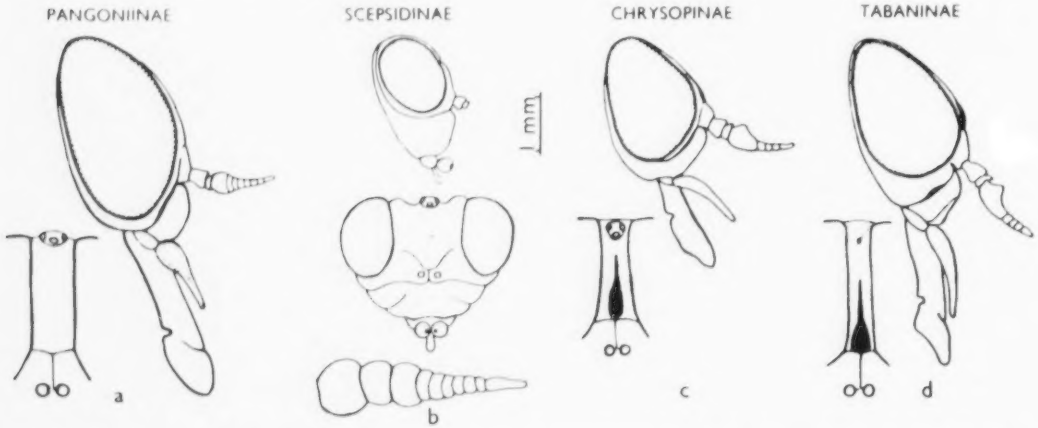

FIG. 2.—Heads and fronts of generalized representatives of subfamilies. a, *Scaptia* (Australia); b, *Scepsis* (Brazil); c, *Veprius* (Chile); d, *Cydistomyia* (Australia). (Adapted with permission from Mackerras, 1954).

evolution in them, there is good general correlation between genitalic and external characters, and the little that is known of the larvae and pupae fits the classification based on the terminalia much better than the old arrangement based on the hind tibial spurs."

Another potentially useful character, which he considered only sparingly, *e.g.*, in *Silvius* and *Chrysops*, because of our yet unsatisfactory knowledge on a world basis, is the varied eye pattern and colors in specialized groups; these were fragmentarily employed by Lutz (1914) and Schuurmans Stekhoven (1926). It seems likely that a broad, systematic study of eye colors will have supplemental value, particularly in Tabaninae and Chrysopinae.

Because of evident convergence, parallelism, intergradation, and frequent exceptions that show up in characters, especially in the more specialized groups, a statement of Mackerras' (1955b) when he complains of infrageneric difficulties in *Mesomyia* could as well apply to many other higher groups: ". . . if subgenera are to be recognized, they will have to be defined, not by single key characters, but by an assemblage of minor characters and trends, which are collectively sufficient to indicate genetic uniformity."

THE OLD AND NEW SYSTEMS COMPARED

In his Genera Insectorum review, Surcouf (1921) treated the family under Loew's (1860) two subfamilies and their contained genera. To accommodate more subfamilies, Enderlein (1925) and Kröber (1934) followed Lutz (1915) in raising the equivalent of these subfamilies to Sections Opistacanthae and Opistanoplae. Though Lutz' (1914) groups, *Tabaninae schistocerae* and *T. haplocerae*, were in a sense the equivalent of tribes, the tribal concept for Neotropical[4] Tabanidae was first applied by Enderlein and refined by Kröber. It was later also adapted to the Nearctic[4] fauna by Philip (1941).

Kröber's (1934) treatment for the Neotropical fauna and Philip's (1947) for the Nearctic may be taken as representative of the "pre-Mackerras era" for comparison with the new in the Western Hemisphere (the equivalent elements in the two faunas are set opposite one another.)

	Kröber (Neotropical)	Philip (Nearctic)
I.	Opistacanthae [section]	Pangoniinae [subfamily]
	Pelecorrhynchinae	[Pelecorrhynchinae, 1941, excluded 1947]
	Silviinae [subfamily]	
	Chrysopini [tribe]	Chrysopini [tribe]
	Silviini	
	Pangoniinae	
	Scionini	[not present]
	Pityocerini	
	Pangoniini	Pangoniini
	[not present]	Merycomyini
II.	Opistanoplae	Tabaninae
	Lepiselaginae	[not present]
	Diachlorinae	Diachlorini
	Acanthocerini	[not present]
	Diachlorini	
	Stenotabaninae	Bolbodimyini
		Stenotabanini
		Chlorotabanini
	[not present]	Chrysozonini
	Bellardiinae	[not present]
	Psalidini	
	Tabaninae	Tabanini
	Tabanini	
	Dichelacerini	[not present]

[4]It is convenient to refer to these Regions in the old arbitrary sense though more modern interpretations, *e.g.*, Townsend (1942), have considerably altered their areas.

As revised by Mackerras' (1954) inclusion of genitalic characters, the systematization of suprageneric taxa of world species contracts to four basic subfamilies with representation in the New World as depicted in the following diagram:

Only two specialized tribes are not represented in the Western Hemisphere, another is found in North America (NA) but not South America (SA), but the largest representation is found in both (Am). One of four small genera of the Scepsedinae is found in Brazil, the remainder in East and South Africa.

Major differences between the above treatment and the arrangements of Kröber and Philip are at once apparent. The Chrysopinae and Tabaninae are conceived as branches of the same stem, and Mackerras (1955a) suggests that new evidence shows some relationship of the small family Scepsidinae to Chrysopinae but that the genus *Braunsiomyia* justifies "maintaining the group as a separate subfamily" (1955b).

The writer's belief that the old, Silviinae and Chrysopinae should be merged is confirmed by Mackerras' genitalic studies and the systematic relationships are greatly improved with deemphasis of the hind-tibial spurs as characters tracing a sort of dichotomous primitive division of the family into two major sections, and more emphasis on the tribal level for concepts of diversification and specialization.

From the zoogeographic standpoint with relation to age in the family, Mackerras remarks that "the tribes of Pangoniinae are certainly the most ancient, those of the Chrysopiinae probably intermediate, and of Tabaninae most recent." It is of interest here to note that the primitive

pangoniine and diachlorine elements are most richly developed in South America with major radiations into the Australasian region, and only one representative of Pangoniini, the genus *Pangonius* is found in the Old World, though a few imperfectly known "Corizoneura"[8] may have scionine relationships. On the other hand, the primitive chrysopine Bouvieromyini have their richest representation in Australia, with "Gondwanaland"-like distribution, and a northern radiation represented in the isolated, peculiar *Merycomyia* of eastern U. S. Though generalized in Mackerras' scheme, the last is actually more specialized than most tabanines in the extreme reduction of the flagellar annuli though the hind-tibial spurs are among the least developed in the subfamily.

In contrast, the more specialized *Chrysops*, Tabanini and Haematopotini have become most successful and prolific in the Northern Hemisphere, the last becoming especially diversified in Africa, and Oldroyd suggests the tribe was once abundant in America. Peculiarly, there is not a single haematopotine representative south of the U.S. in the Americas or east of Java in the South Pacific. As a good example of convergence, however, the peculiar *Dasybasis argentina* (Brèthes) from southern South America shows such remarkable resemblance to the haematopotine "water marked" wing pattern, even to the suggestion of rosettes and a marginal band, that it was described in *Chrysozona* (= *Haematopota*) originally; the subepaulets have remained bare, however, and the style four-annulate.

Several species of *Chrysops* and *Hybomitra* are holarctic, and *Haematopota champlaini* (Phil.), rare in eastern U.S., is exceedingly close to the common *H. pluvialis* (Linn.) of the Old World (Philip, 1953). It appeals to me that the Haematopotini may have derived very recently in the Palaearctic Region and included the Nearctic among its secondary radiations, in a sense retracing the ancestral migratory route as *Hybomitra aequetincta* (Becker) appears to have done.

It is peculiar that no tabanid has become successfully established in Greenland. Nor are any holantarctic tabanid species known.

While Mackerras has done a remarkable job in sampling the world's tabanid fauna in establishing and revising basic suprageneric relationships, his work also emphasizes need for intensive, complementary studies by students of the several continental faunas. In South America this need is particularly evident in Diachlorini and in North America in Chrysopini. In regard to the latter, Mackerras' (1955b) remarks about *Mesomyia* and its relatives could almost equally apply, *viz.*, "This decision [subgenera vs. genera] can only be personal and arbitrary, depending on the weight the individual workers give to particular characters."

Among the generalized Pangoniini in western North America, the utility of his genitalic studies was strikingly illustrated in reassessment of species assignable to the closely related *Stonemyia* and *Pilimas*, the latter originally described as a subgenus of the former, illustrating that external similarities are not necessarily also reflected in genitalic agreement. It is obvious that more attention will have to be paid to genitalic structures in Tabanidae, though it is also apparent that these will never reveal the fine specific characters such as are now so indispensable for students of the Culicidae and Simuliidae.

[8]It is probable that *Corizoneura yezoensis* Shir. of Japan is a *Stonemyia*, the first known of these species outside North America, as called to my attention by L. L. Pechuman (correspondence). A female from Nikko differs from dark *S. tranquilla fera* (Will.) only in narrower pale abdominal incisures, shorter proboscis, and in that the body hairs are not as yellow. *Corizoneura chekiangensis* Ouchi from eastern China is likely a *Stonemyia* also. However, genitalic studies of both sexes of these are needed in view of Mackerras' statement (1955a) that "it would be wise to leave a final decision on the status of *Stomemyia* and *Pilimas*, until the Palaearctic species of *Scaptiella* and '*Corizoneura*' have been re-examined."

SUMMARY

Revision of the higher systematics of the Tabanidae of the Americas is discussed in the light of new methods of approach, particularly by correlation of genitalic characters as introduced by Mackerras' studies of the world fauna. Representative genera in all four revised subfamilies in the new arrangement and all but two specialized tribes, are found in parts of the Western Hemisphere.

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DEVELOPMENT, STRUCTURE, AND FUNCTION OF COVERING OF EGGS OF FLOODWATER MOSQUITOES. I. OVARIAN DEVELOPMENT[1]

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There are a number of readily observed phenomena associated with the eggs of floodwater mosquitoes that suggest the shell to be a structure with unusual properties. In North America these eggs are produced by mosquitoes in the genera *Aedes* and *Psorophora*. They are laid in situations where conditions of transient water occur, retaining their viability out of water in nature for a year or longer. Inasmuch as a fully developed embryo is within the egg during such extended periods (in contrast to the partially developed embryo within diapause eggs of grasshoppers), it seems obvious that factors tied up with successful hatching are connected with the egg coverings. It has been shown that oxygen must be removed from the surrounding medium before hatching will take place, but even when such a condition exists the hatching will be irregular if certain other conditions have not been met first (Horsfall, 1956). These environmental conditions differ among species, and have been collectively termed "conditioning" by Borg and Horsfall, 1953. While the determination of structure and composition of the chorion may not clarify factors responsible for the observed phenomena, such findings are a desirable adjunct to further study.

A comparative study of the structure of chorionic layers of several species of floodwater mosquito eggs suggests a useful method of determining their function. A further method of value is to compare the structure of eggs of floodwater mosquitoes with the eggs of mosquitoes that are not generally durable (for example *Anopheles* and *Culex*). Fully formed biological structures are often best understood by first studying their development. The ovarian development of eggs of floodwater mosquitoes is recounted here because this has not been adequately described previously. The layers of the shell, and some of the humidity relations of complete eggs will be discussed subsequently. Pertinent aspects of the previous findings in eggs of insects, and specifically of mosquitoes, are also related here.

REVIEW OF LITERATURE

Determination of the layers in egg coverings has been undertaken principally with larger eggs that can be sectioned readily. The chorion can usually be divided into two subdivisions called the exochorion and endochorion (Wigglesworth, 1950). Further subdivisions appear to vary between species, as many as seven strata being recorded by Beament (1946) in *Rhodnius* eggs.

The structure of coverings of eggs of mosquitoes has been best described at the time of development when the chorion becomes patterned. Development of the coverings has been studied in *Culex* by Nath (1924) and Christophers (1945). Nicholson (1921) has described the development of coverings of the eggs of *Anopheles*. Bauer (1933) examined the development of the oocyte nucleus in *Aedes aegypti* (L.), but other structures of the egg were not described in detail. Möllring (1956) mentioned the fact that developing eggs of *Aedes* possess seven nurse cells. He briefly described the structure of the nurse cells, and the oocyte nucleus during its early stage of development.

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[2]A portion of a thesis submitted in part as the requirement for the PhD. degree in entomology at the University of Illinois, Urbana, Ill.

Accounts of the structure of the chorion of eggs of floodwater mosquitoes have been limited to descriptions of the hexagonal print left on its surface by cells of the follicular epithelium. The specificity of this surface pattern has been used by Horsfall *et al* (1952) to distinguish the eggs of seven species of *Psorophora*. Similar specificity was used by Horsfall and Craig (1956) to determine the eggs of *Aedes* common in Illinois.

The best description of the exochorion of a mosquito is that of de Buck (1938) for *Aedes aegypti*. Exochorion covers the entire surface of eggs of *Culex* and of floodwater mosquitoes, but rarely covers more than a part of the surface of eggs of *Anopheles*. Exceptions are the so-called winter eggs of some species of *Anopheles* (Hurlbut, 1938, *Anopheles walkeri* Theo.). The exochorion of *Culex* has been illustrated by Christophers (1945) as being composed of two membranes, with projections based on the inner and passing through the outer membrane. An oily layer is claimed by the same author to be present in the exochorion of *Culex*.

Little has been determined about the chemical nature of the chorion. Earlier workers described it as being "chitinized" (Atkin and Bacot, 1917, *Aedes aegypti*) but Nicholson (1921, var. *Anopheles maculipennis*) recognized that although it superficially resembles chitin, it is soluble in warm solutions of potassium hydroxide. Several investigators (Herms and Freeborn, 1920, *Anopheles* spp.; Nicholson, 1921, var. *Anopheles maculipennis* (Meig.); and Gander, 1951, *Aedes aegypti*) mentioned the transition of chorion from a soft white envelope at the time of oviposition, to a dark and sclerous one within an hour. Gander (1951) stated that this change in color and texture can take place in eggs of *Aedes aegypti* under paraffin oil, and he consequently believed a tanning process was involved.

Orientation of chorionic layers has not been clearly demonstrated in mosquito eggs. Christophers (1945) pointed out that the cap of *Culex* does not become completely separated, but remains attached by a narrow unbroken part of the chorion which is always situated in the middle of the dorsal aspect, corresponding to the position of the hatching spine of the embryo. Jones and Arnold (1952) mentioned that in *Aedes nigromaculis* (Ludlow) abnormal larval hatching may take place, with the break in the chorion being in the form of a longitudinal split.

The permeability of chorion is not precisely known. Chorion of freshly laid eggs is said to be permeable to water at the time of oviposition, osmosis accounting for the increase of size noted during the period prior to darkening (Davis, 1928, *Anopheles* spp.; Downs, 1951, *Anopheles* spp.; and Gander, 1951 *Aedes aegypti*). Powers and Headlee (1939) believed the chorion to be impermeable to oils, and the ovicidal effect of oils to be due to prevention of respiration of oxygen. Buxton (1932) felt that resistance of eggs to desiccation was due to actual ability to retain water, because if eggs shriveled they could not be made to hatch. It was shown by Gander (1951) that eggs of *Aedes aegypti* became resistant to desiccation only at about fifteen hours following oviposition. It is possible that the impermeability of chorion accounts for the difficulties encountered by Hayes (1950) in achieving significant mortality of embryos of *Aedes* treated with DDT. Such resistance to the effect of insecticides was also noted by Downs (1951) in *Anopheles* eggs. The impermeability of chorion has been the main deterrent to a good description of the embryology of mosquitoes, although it is claimed that this difficulty has been overcome (Beckel, 1953).

Factors contributing to rate and uniformity of hatching have been noted but not clearly understood. Roubaud (1929) ascribed the discrepancies observed in the rate of hatching eggs of *Aedes aegypti* to the presence of two types of eggs. Those that hatched readily were termed "active" eggs, and those that hatched after a long period of flooding were called "subactive" eggs. Hovanitz (1946) asserted that variations in the frequency of hatching of eggs of *Haemogogus* indicated a quality imposed through the female. Eggs of *Aedes aegypti* were noted in the laboratory to hatch more readily when moistened for several hours before submergence in water (Shannon and Putnam, 1934; Burchfield *et al.*, 1953). The

EXPLANATION OF FIGURES IN PLATE I

FIG. 1. Section of ovary of *Psorophora ciliata* at time of feeding on blood. × 211. E, epithelium of ovariole; LO, lateral oviduct; N, nucleus of nurse cell; O, nucleus of oocyte; PS, peritoneal sheath; TR, trachea; V, vitellarium or egg chamber.

FIG. 2. Section of egg chamber of *P. ciliata*, 10 hours after feeding on blood. X 419. E, epithelium of ovariole; N, nucleus of nurse cell; O, nucleus of oocyte.

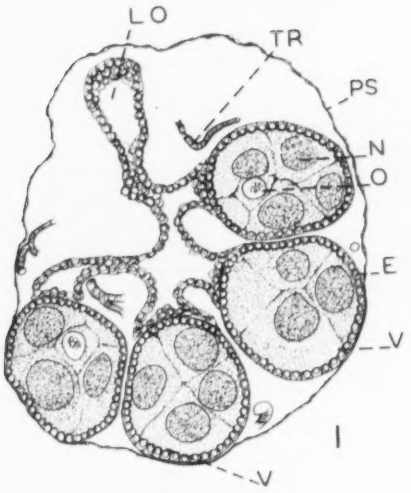
FIG. 3. Section of vitellarium of *Psorophora ciliata*, 24 hours after feeding on blood. × 350. E, epithelium of ovariole; N, nucleus of nurse cell; O, nucleus of oocyte; Y, yolk granules. Cell membrane of oocyte has disappeared and cytoplasm appears continuous with developing yolk. Borders of nucleus of oocyte very irregular, prior to dispersion.

FIG. 4. Section of vitellarium of *P. ciliata*, 34 hours after feeding on blood. × 277. C, chorion; E, epithelium of ovariole; N, nucleus of nurse cell; Y, yolk. Nucleus of oocyte completely scattered. Note chorion lacking in region of nurse cells.

FIG. 5. Section of anterior end of vitellarium of *Psorophora ciliata*, 58 hours following feeding on blood. × 211. C, chorion; E, epithelial cells surrounding developing egg; N, nucleus of nurse cell; VA, vacuole; Y, yolk. Note absence of chorion in region of nurse cells.

FIG. 6. Section of anterior end of vitellarium of *P. ciliata*, 72 hours following feeding on blood. × 211. C, chorion; E, epithelial cells around developing eggs; NE, combination of extruded nurse cells and epithelial cells; Y, yolk.

FIG. 7. Detail of formation of spines in exochorion of *Psorophora ciliata*, prior to 81 hours following feeding on blood. × 211. C, chorion; E, nucleus of cell in epithelial layer; SP, spine; Y, yolk. Great expansion of chorion is due to fixation with alcoholic Bouin's.



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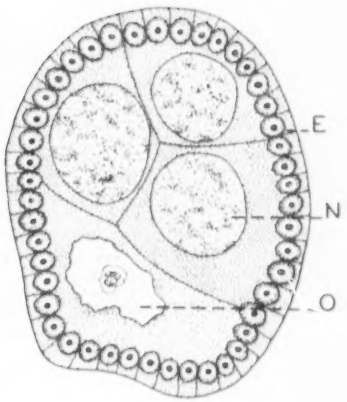

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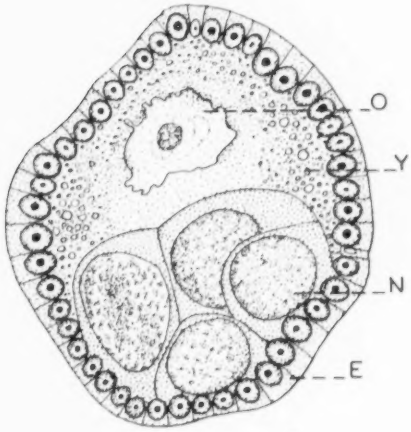

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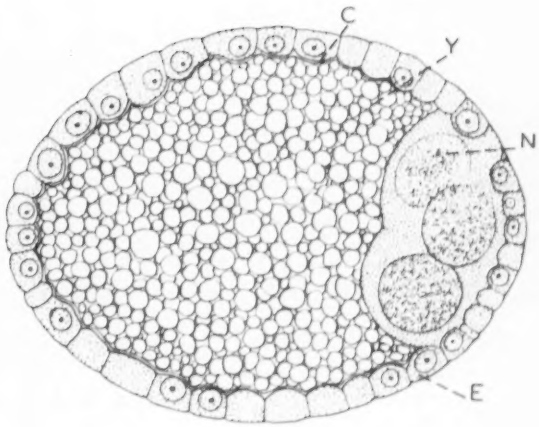

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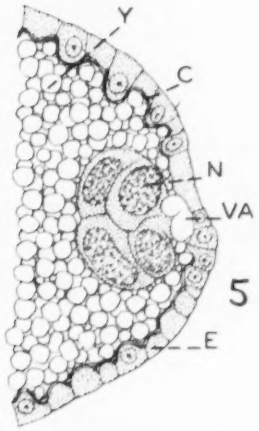

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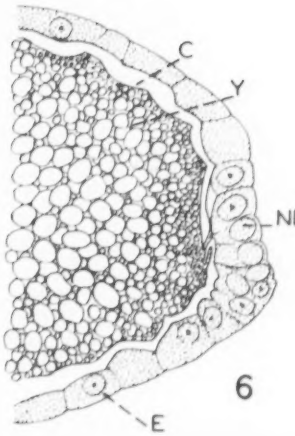

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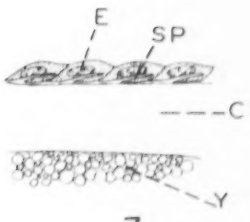

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necessity of "conditioning" for uniformity of hatching under identical conditions of submergence was emphatically stated by Borg and Horsfall (1953), who showed that the stimulus for hatching of conditioned eggs is reduction in amount of oxygen in the medium.

MATERIALS AND METHODS

Comparisons were made of the developing eggs of a number of floodwater mosquitoes. Observations on events during development were begun with wild adult females captured in the act of biting. These were fed simultaneously, and the ovaries dissected out at timed intervals following a meal of blood. Two species, *Aedes aegypti* (Linn.) and *Culex pipiens fatigans* (Wied.), were reared in the laboratory for supplementary studies. Females which had fed on blood were held under conditions of low light intensity, high humidity, and moderate room temperature (28°C.) in order that conditions during ovarian development might be as standard as possible.

Dissections were made in 0.9 percent saline, and the ovaries were prepared by conventional procedures of microtomy. The ovaries were fixed in Bouin's fixative for 6 to 25 hours immediately after excision. In later stages, when chorion had become brittle and impermeable, fixation was accomplished in alcoholic Bouin's for 23 to 33 hours. Washing of fixatives and dehydration by a graded series of alcohol was accomplished in the standard manner. Benzene was found to favor good infiltration of the 56° to 58°C. paraffin used for embedding.

Thin sections were most desirable for examining structural details, and certain refinements of technique aided such preparations. The microtome knife, used in conjunction with a rotary microtome, was carefully sharpened on a glass wheel. Rapid manipulation allowed continuous sectioning at three microns. The ribbons were affixed with gelatin and formalin and subsequent hydration, staining, dehydration, and mounting in balsam, were performed in the standard manner. Most of the sections were stained with haemalum and counterstained with eosin. Some specimens were mounted unstained for examination with the polarizing microscope.

The ovarian development of eggs of floodwater mosquitoes, for which *Psorophora ciliata* (Fabr.) is here used as a model, is essentially the same for all species examined. *Aedes aegypti*, *A. stimulans* (Walker), *A. vexans* (Meig.), *A. trivittatus* (Coq.), *Psorophora ferox* (Humboldt) and *P. horrida* (Dyar and Knab) were also studied. Since eggs of *Culex* do not tolerate drying, their development in *Culex pipiens fatigans* was compared. Despite the major differences in the physical properties of durable and non-durable eggs, differences in ovarian development mainly apply to size and minor structural details. Illustrations of developmental stages were made with the aid of a squared ocular in the compound microscope.

OVARIAN DEVELOPMENT

The egg nearest the oviduct within each ovariole is the most advanced in development. This advanced terminal egg is referred to whenever time is related to the development of structures. At the time the descriptions begin, the most advanced eggs have completed their development just prior to the formation of yolk. This is the state of development usually present in female mosquitoes in the act of biting.

Epithelial cells connected with the development of eggs line the ovariole at the time the adult feeds on blood, consequently enlargement of eggs is due to the elaboration of non-cellular structures by the cells. Where new structures appear at the time of disappearance or reduction in size of cells associated with developing eggs, it is assumed that such cells play a major role in the formation of the new structures.

At the start of development, each egg chamber or vitellarium contains an oocyte and seven nurse cells (fig. 1). At this time the two types of cells are readily distinguished because the nuclei of nurse cells are larger and stain more readily than the nucleus of the oocyte. The condition of nurse cells and oocyte being contained in a vitellarium is common among Diptera, and is referred to as meroistic and polytrophic.

With the progression of time the nucleus of the oocyte disappears from view. Following a blood meal it increases in size, and its borders

EXPLANATION OF FIGURES IN PLATE II

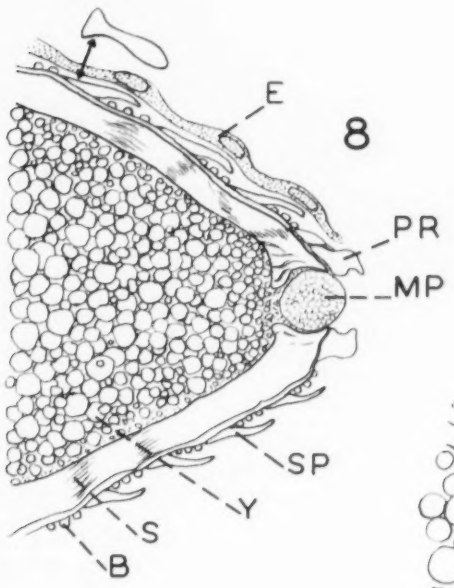
FIG. 8. Section of anterior end of fully developed egg from ovary of *P. ciliata*, 81 hours after feeding on blood. × 320. B, boss of exochorion; E, layer of epithelial cells; MP, micropylar plug; PR, pellicular ring; S, stratification of chorion; SP, spine; Y, yolk. Chorion thicker than normal is due to fixation with alcoholic Bouin's. Note breaks in chorion around micropylar plug caused by brittle nature during sectioning. Spine of upper portion shown in surface view.

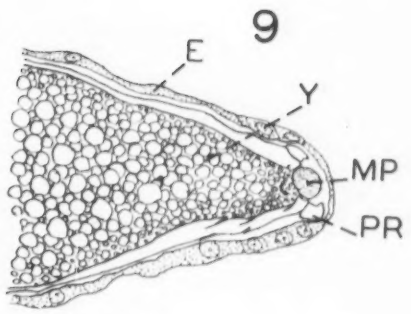
FIG. 9. Section of anterior portion of fully developed egg of *P. horrida*, 73 hours after feeding on blood. × 320. E, ovarian epithelium; MP, micropylar plug; PR, pellicular ring; Y, Yolk. Chorion somewhat expanded due to fixation with alcoholic Bouin's. Note lines of fissure in chorion caused by sectioning.

FIG. 10. Section of anterior end of fully developed egg from ovary of *Aedes vexans*, 109 hours after feeding on blood. × 896. C, chorion; E, epithelium; MP, micropylar plug; Y, yolk.

FIG. 11. Section of anterior end of fully developed egg from ovary of *A. stimulans*, 121 hours after feeding on blood. × 420. C, chorion; MP, micropylar plug; NE, combination of extruded nurse cells and epithelial cells; V, vacuole; Y, yolk.

FIG. 12. Section of anterior end of fully developed egg from ovary of *Culex pipiens fatigans*, 70 hours following feeding on blood. × 840. C, chorion; EX, exochorion; MA, micropylar apparatus; MP, micropylar plug; Y, yolk.


E
8
PR
MP
SP
Y
S
B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E
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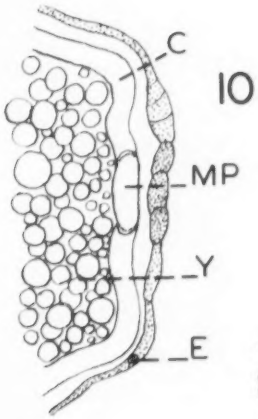
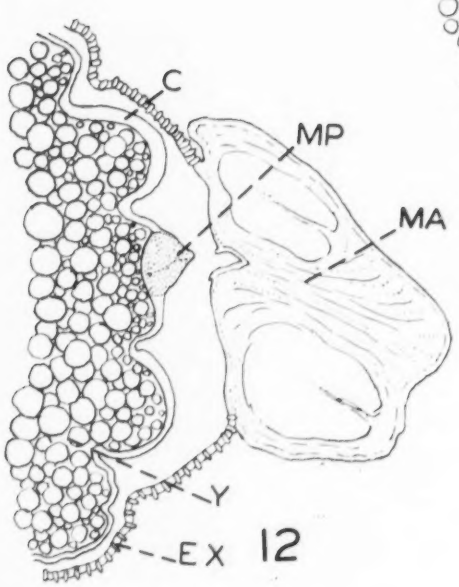
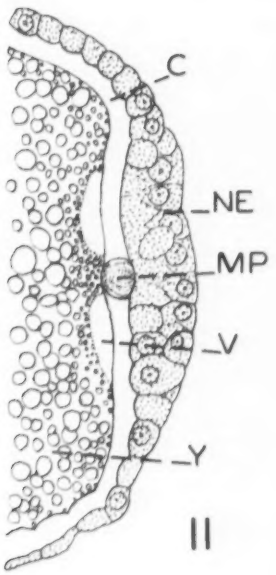

C
10
MP
Y
E


C
MP
MA
Y
EX
12


C
NE
MP
V
Y
11

become irregular in outline (fig. 2, 3). Some 34 hours following a blood meal, no traces of the nucleus are visible with the stains used. It is assumed that the disposition of the nucleus of the oocyte of *P. ciliata* is similar to that found by investigators who have examined the oocytes of other species of mosquitoes. Prominent among such records are those of Bauer (1933) who reported on *Aedes aegypti*, and on the variants of *Anopheles maculipennis* (Meig.); and Nicholson (1921) who reported on a variant of *Anopheles maculipennis* and *Culiseta annulata* (Schrank). In all these species the nucleus branches throughout the cytoplasm and finally becomes so dispersed that it cannot be seen among the yolk granules. In addition to obscuring the nucleus of the oocyte, the rapid addition of yolk granules increases the size and changes the shape of the egg. The originally spherical vitellarium becomes increasingly elongate.

The seven nurse cells initially present within each vitellarium are extruded through the anterior end of the egg. While still in the confines of the egg their nuclei stain readily and contain a homogeneous plasm (figs. 1, 2, 3, 4, 5). Prior to extrusion the nurse cells serve as a landmark for the anterior end of the egg. After extrusion, nuclei of the nurse cells become greatly reduced in size and are not readily distinguished from those of the epithelium among which they lie (fig. 6).

Chorion, which is lacking during early stages of development, is secreted by the epithelium around the developing egg. It is just visible at 34 hours (fig. 4) and is present over the entire surface of the egg with the exception of the region of the nurse cells (figs. 4, 5). For the most part chorion seems homogeneous, or granular in early stages of formation. This granularity, like the elastic separation of developing chorion sometimes observed, is probably a result of faulty fixation. As chorion increases in thickness it appears very faintly striated (figs. 5, 8) and the color of dark amber. Thicker chorion on being fixed for sectioning becomes so brittle and impermeable that a more penetrating fixative such as alcoholic Bouin's is required. Usually this fixative distorts the chorion by causing it to swell. Cells of the follicular epithelium leave their outlines on the chorion, with a raised border between adjacent cells.

The exochorion is the outermost envelope of the egg. It is secreted by the epithelium following deposition of the chorion. There is an inner or basal membrane, and an outer one separated by small projections or bosses. The membranes and bosses are secreted at the surface of the epithelial cells and are not readily seen in correct relationship in sections of ovarian eggs. In species of *Psorophora* a spine ("projection of pellicle" of Horsfall *et al.*, 1952) is formed within each epithelial cell near the nucleus (fig. 7). The spines are slightly curved and directed anteriad, being pointed at the tip when viewed from the side (fig. 8). In surface view these tips are flattened and bluntly rounded. A special modification of material similar in appearance to that composing the spines is present in the form of a raised ring around the region of the micropyle (fig. 8). This is the "pellicular ring" of Horsfall *et al.*, (1952).

The epithelial cells, which are active during the secretion of the chorion and exochorion, degenerate by the time the egg is fully developed. This single layer of cells which surrounds the developing egg is readily recognized by its nuclear appearance. The nucleus of each cell is well defined and contains a centrally located endosome until the time of degeneration. Mitotic figures were not seen within the epithelium, accordingly the adjustment to the growing egg is accomplished by the enlargement of individual cells rather than an increase in their number. Upon completion of development of the egg, the epithelial layer degenerates to the point where it is present only over the anterior end. At the anterior or micropylar end, epithelial and extruded nurse cells combine in a specialized region similar to the "rosette cells" described by Nicholson (1921) in *Anopheles maculipennis*. In *P. ciliata* this group of cells lacks the uniformity of numbers and design characteristics of rosette cells. At the final stage of development, cells of the rosette-like region have degenerate nuclei and ill defined borders (fig. 11). Apparently this specialized group of cells forms a plug which, because of its implied importance, merits a more detailed description.

The micropylar plug is a mass of material in the micropyle of the egg. It is composed of a substance similar in appearance to chorion but is usually a trifle darker and appears finely granular rather than striated. It so fills the micropylar opening that this opening is difficult to delimit. This mass is the "stopper" that Nicholson (1921) found in *Anopheles maculipennis*, and the "egg spike" that Christophers (1945) described in the egg of *Culex pipiens*. Because of its location and probable function, the term micropylar plug is preferred to the above terms.

The micropylar plug is necessarily described from its structure in fully developed eggs within the ovary. In eggs which have been laid it is most readily visible in the detached apical end or cap of hatched eggs of large species of *Psorophora*. These empty shells, when prepared as water mounts, show the micropylar plug as a granular region in the anterior tip. In species of *Psorophora* the micropylar plug projects above the general surface of the chorion forming the "micropylar mound" described by Horsfall *et al.* (1952). The minute aspects of the micropylar plug were not examined with the aid of the electron microscope because, although numerous attempts were made, no ultrathin sections of this object were obtained.

Minor differences are apparent in the micro-

pylar plugs of various species of floodwater mosquitoes. In *Psorophora ciliata* (fig. 8) the micropylar plug protrudes slightly above the outer surface of the chorion in which it is embedded. The plug is clearly distinct from the chorion although they are of about the same width. Structurally the plug appears granular and lacking in orientation. The micropylar plug of *P. horrida* (fig. 9) is similarly embedded, but it is thicker than the chorion. Weakly visible lines of striation run from the outer to inner surface of the plug in this species. In *Aedes vexans* (fig. 10) the micropylar plug has a considerable surface area and is flush with the chorion. Its texture is not discernible. The plug of *Aedes stimulans* (fig. 11) appears minute and nearly surrounded by the chorion. Vertical striations are visible within it. In contrast to those of floodwater mosquitoes, the micropylar plug of *Culex pipiens fatigans* (fig. 12) has a large exposed surface area, is much wider than the surrounding chorion, and is not readily discerned as being distinct from the chorion by its color or its borders.

SUMMARY

The developing envelopes surrounding eggs of floodwater mosquitoes were examined, using conventional procedures of microtomy, and microscopy with non-polarized light. With these methods the following points were determined:

1. The cellular development and mode of secretion of envelopes are essentially the same as has been described in *Anopheles* and *Culex*.

2. A structure occluding the micropyle, termed here the micropylar plug, differs from that found in *Anopheles* and *Culex*. The form and position of this plug in the eggs of floodwater mosquitoes suggest that it plays a role in preventing loss of water from the egg.

3. At the time of oviposition the egg is covered by an outer region, the exochorion, and an inner layer, the chorion. At this time no inner layer such as a vitelline membrane could be demonstrated.

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THE COMPARATIVE INTERNAL LARVAL ANATOMY OF SEVERAL GENERA OF SCARABAEIDAE (COLEOPTERA)[1]

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While considerable work has been done on the internal larval anatomy of Hymenoptera, Diptera, and Lepidoptera yet, so far, little has been published on the internal larval anatomy of Coleoptera. Recently, workers have demonstrated the value of the internal larval anatomy as a taxonomic tool and as an indicator of phylogenetic relationships. The work that has been done on the comparative internal larval anatomy of sawflies is an example. Yuasa (1932, pp. 120–132) stressed the intrinsic importance of the immature stage in a study of the phylogeny of sawfly larvae. He also recognized the danger of mistaking adaptive structures for phylogenetic indices. Maxwell (1955) had considerable success in using the internal anatomy of sawfly larvae for studying phylogenetic relationships. Moreover, the internal anatomy can be used in determining sawfly larvae to genus and to species as well as to family and subfamily.

In the present phylogenetic studies in the family Scarabaeidae, the internal anatomy of the larvae of four genera representing three subfamilies was compared. The family Scarabaeidae was chosen because it is considered by writers to be the most primitive family of the superfamily Scarabaeoidea. In making these studies the phylogenetic scheme of subfamilies of Leng (1920, pp. 248–264) is tested by comparing the digestive and nervous system of these larvae: *Pleocoma crinita* Linsley of the subfamily Pleocominae; *Lichnanthe rathvoni* Lec. of the subfamily Glaphyrinae; *Polyphylla decemlineata* Say and *Dichelonyx lateralis* Fall of the subfamily Melolonthinae.

The internal anatomy of different larval instars of each genus is also compared. Seven instars of the genus *Pleocoma* are compared.[3] In the other genera which have only three instars, the second and third instars are compared. These several comparisons were made to determine which changes were associated within the species and which changes were associated with generic differences.

ACKNOWLEDGMENTS

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The writer is also indebted to Mr. Floyd Ellertson, research assistant of the Mid-Columbia Branch Experiment Station, who identified the stages of *Pleocoma* which were used in this problem.

MATERIALS AND METHODS

Living and preserved larvae of different instars were studied. Dissections were carried out under a dissecting microscope with magnifications of 14 to 60 X. Injection with trypan blue in saline solution was useful in demonstrating the outline of digestive tracts and gastric caeca. For gross dissections, specimens were dissected and pinned out under 70% alcohol. Within a few hours, such delicate structures as the ventriculus and gastric caeca became sufficiently hardened for dissection. For studying the movement of the gut, specimens dissected in 0.9 normal saline could be observed for at least half an hour. Nervous systems were studied by dropping four or five drops of Bouin's solution on the dissected specimens so as to distinguish the brain, ganglia, and nerves from other parts of body. A number of larvae were dissected for each category studied in order to learn the degree of variation.

Both transverse and longitudinal serial sections were made for the digestive tracts of all the genera studied in order to compare similarities and differences between genera, such as the formation of muscles, epithelia or any other particular characters. Standard staining techniques were followed by fixing the material in Bouin's fluid, imbedding in paraffin, sectioning, mounting, and staining with Harris's hematoxylin and eosin.

DIGESTIVE SYSTEM OF SCARABAEIDAE

Digestive Tract Changes for Instars of Pleocoma

The changes occurring in the alimentary canal of *Pleocoma* larvae were observed for several instars. According to data furnished by Mr. Floyd Ellertson and Dr. P. O. Ritcher, head

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[3]Mr. Floyd Ellertson and Dr. P. O. Ritcher state that *Pleocoma* is unusual in having many instars while other Scarabaeidae normally have only three instars. (Personal Communication).

capsule measurements give a rough approximation of what instar was being observed. Table I presents the head-capsule data.

TABLE I

HEAD CAPSULE MEASUREMENTS OF PLEOCOMA SP. LARVAE OBSERVED AND THE APPROXIMATE INSTAR

WIDTH OF HEAD CAPSULE	INSTAR
2.2 mm.	1
3.43 mm.	4
5.00 mm.	6
6.43 mm.	8
7.14 mm.	9 (last instar)
7.86 mm.	10 (last instar)
8.30 mm.	11 (last instar)
8.73 mm.	11 (last instar)

The initial observation was made when the first instar *Pleocoma* larvae were 2 days old and again when they were 10 days old. The exact age of the others was unknown.

No major changes occurred in these larvae except in changes of the gut-length and in the development of the gastric caeca. In the 2- and 10 day old larvae of the first instar, there is no major change in the length of the fore- and mid-gut (fig. 1) for the 8 day interval. In the first instar the hind-gut was a little longer than the other parts. Two sets of gastric caeca were present at the anterior end of the mid-gut. The first set appears on the ventral side as swellings which are blind pouches. The second set is located just posterior to the first set and appears as small annuli around the gut.

In the fourth to the eighth instars of *Pleocoma* the fore- and hind-gut remain nearly of the same length while the mid-gut is about twice as long as the fore-gut (fig. 2). The first set of gastric caeca branches into five lobes covering the underside of the crop while the second set develops into small blind pouches (fig. 5).

In the late instars, both sets of gastric caeca degenerate. At the same time the mid-gut shortens while the fore- and hind-gut elongate. In these late instars, fore- and hind-gut are nearly the same length while the mid-gut is only one-half as long (fig. 4). The elongation seems to occur at the posterior end of the fore-gut and anterior end of the hind-gut. The malpighian tubules move anteriorly to a point just posterior to the pyloric valve. The adult stage has no gastric caeca.

The change in the length of the gut and the development of gastric caeca are probably adaptations for the feeding of the larvae. Sections of the alimentary canal showed no other changes except the number of the epithelial cells is tremendously increased. Active secretion seems to be great in the mid-gut and even greater in gastric caeca during the feeding stages. The epithelial cells are reduced and become degenerated in late instar larvae. This condition may indicate that the late instar larvae do not feed.

The alimentary canals of the second and the third instars of the genera *Lichnanthe*, *Dichelonyx* and *Polyphylla* showed no differentiation.

Description and Comparison of the Alimentary Canals of Scarabaeid Larvae

Length.—The length of the alimentary canal of both adult and larvae scarabaeid insects is known to be correlated with their feeding habits. Becton (1930, p. 315) studied the digestive tract of adult *Phanaeus vindex* Macl. (Coprinae) which feed on dung and found that the tube is of great length, averaging a little less than eight times the length of the body. Swingle (1950, p. 185) in studying the digestive tract of adult Japanese beetles (*Popillia japonica* Newm.: Rutelinae) which feed on fresh leaves, found that it is approximately twice as long as the body. Rapp (1947, p. 145) found the larval digestive tract of *Canthon pilularius* (L.) (Coprinae), which feed on dung to be about twice the length of the body. In the case of *Oryctes rhinoceros* (L.) (Dynastinae) it is only a little longer than the body in the third larval instar (Gressitt, 1953, p. 24). In the alimentary canal of the larvae which were studied here, there are only apparently minor differences in the length, ranging from 1.3 to 1.8 times the length of the body.

Fore-gut.—The fore-gut of most of the adult scarabaeid insects is very short when compared with other parts of the canal. This is shown in the cases of adult *Phanaeus vindex* (Becton, 1930, p. 135), *Popillia japonica* (Swingle, 1950, p. 185). Fletcher (1930, p. 110) and Jones (1940, p. 95) found that in the adult of *Phyllophaga gracilis* (Burm.) and of *Diplotaxis liberta* (Germ.) the fore-gut comprised about 12% and 10% of the total length respectively. This is also true in the larvae which were studied here. The fore-guts of *Pleocoma*, *Lichnanthe*, *Dichelonyx* and *Polyphylla* are about 25%, 12%, 10%, 10% respectively, of the total length of the body.

The fore-gut extends from the mouth in the head to the first or second thoracic segment. It is not highly specialized, and its major function probably is no more than transferring food from the mouth to the mid-gut. All the larvae which were studied had a narrow tube-like fore-gut (figs. 2, 11, 12, 13). Histologically the fore-gut shows no difference from that of a typical insect. The outer layer consists of very conspicuous and numerous circular muscles which surround the inner layer of less numerous longitudinal muscles. The epithelial cells have an indistinct basement membrane and usually follow the longitudinal folds of the membrane which lies on the inner surface of the circular and longitudinal muscles (figs. 23, 24, 25). The innermost layer is of chitin which is homologous with the cuticula of

the body wall, and is known as the intima. This intima forms a rather thin, non-cellular, almost transparent layer which lines the entire fore-gut and is more or less thrown into longitudinal folds corresponding to the epithelial layer.

Pharynx.—The initial region of the digestive tract just posterior to the mouth, called the pharynx is evident as a short, slight dilation of the tract in the head. The external circular muscles and intima are thicker than other parts of the fore-gut (fig. 23). The intima may be covered with chitinous spines as found in *Pleocoma* and *Dichelonyx* larvae or the spines may be absent as in the cases of *Lichnanthe* and *Polyphylla*. These spines are also found in the adult stage of *Phanaeus* (Becton, 1930, p. 316), *Phyllophaga* (Fletcher, 1930, p. 111), *Diplotaxis* (Jones, 1940, p. 96), *Popillia* (Swingle, 1950, p. 185). Spines, when present, usually protrude inwardly and caudally (fig. 42). The epithelial cells are cuboidal in shape and line the numerous folds of the walls of the pharynx. Such folds appear where the lumen constricts at the posterior end of the pharynx, begins. The longitudinal muscles are not well developed except in the pharynx of *Pleocoma*.

Oesophagus.—The oesophagus is marked by the constriction of the tube between the pharynx and dilated tube of the crop. No distinct division of this part can be drawn. In this portion the circular muscles and the innermost intima are thinner than in the pharynx region. The longitudinal folds are well developed especially in *Lichnanthe*, so that the lumen of the oesophagus is very narrow. These folds are formed by cuboidal epithelial cells and the intima. Spines are not found in this region.

Crop.—The crop is present as a slight dilation of the posterior portion of the oesophagus, but is different from that of many insects in that it is not large and conspicuous. It is also present in the larva of *Oryctes rhinoceros* (Gressitt, 1953, p. 24). It is doubtful whether it functions as a food reservoir as true crops do. Histologically, the crop consists of very thin circular muscles and the intima. These form the thinnest part of the fore-gut (fig. 24). The internal longitudinal muscles are underdeveloped. Some isolated strands of longitudinal muscles extending from the mid-gut insert at the distal end in the external circular muscles. The basement membrane is indistinct. The epithelial cells are of a low cuboidal shape. Spines and other special structures are not found on the intima.

The stomodeal or cardiac valve is found at the junction of the fore- and mid-gut. This structure is formed by the slight extension of folds of the fore-gut into the lumen of the ventriculus. The valve is composed of the epithelial cells and the intima of the fore-gut (figs. 43, 44, 45, 50). These cells become longer and longer in shape as they extend along the innermost side of the fold. Then they decrease in size where the projection of the fore-gut becomes folded back upon itself and passing forward unites with the mid-gut wall. The intima which is continuous around the valve, stops at the point where the fore- and mid-gut are united. No special structures such as proventricular teeth, pads or spines have been found in these larvae.

Ventriculus.—The mid-gut is an elongate tube which is marked anteriorly by the cardiac valve, and posteriorly by the pyloric valve. This part is usually very long and is a rather large division of the digestive tract. Becton (1930, p. 316) found it 6.7 times as long as the body in adult *Phanaeus*. It comprises more than one-half of the total length in adult *Phyllophaga* (Fletcher, 1930, p. 110) and *Diplotaxis* (Jones, 1940, p. 95). In larvae the length varies as follows: in *Pleocoma* it comprises about 50%, *Lichnanthe* 30%, *Polyphylla* about 30% and *Dichelonyx* 40% of the total length. It is a fairly straight tube, tapering slightly from the anterior to the posterior end and extending from about the first thoracic segment to the eighth abdominal segment.

Special structures may be found in the mid-gut of some scarabaeid insects. Crypts or papillae occur in the mid-gut of adult *Phanaeus* (Becton, 1930, p. 316) and *Passalus* (Lewis, 1926, p. 18). Such structures are not found in the larvae which were studied here.

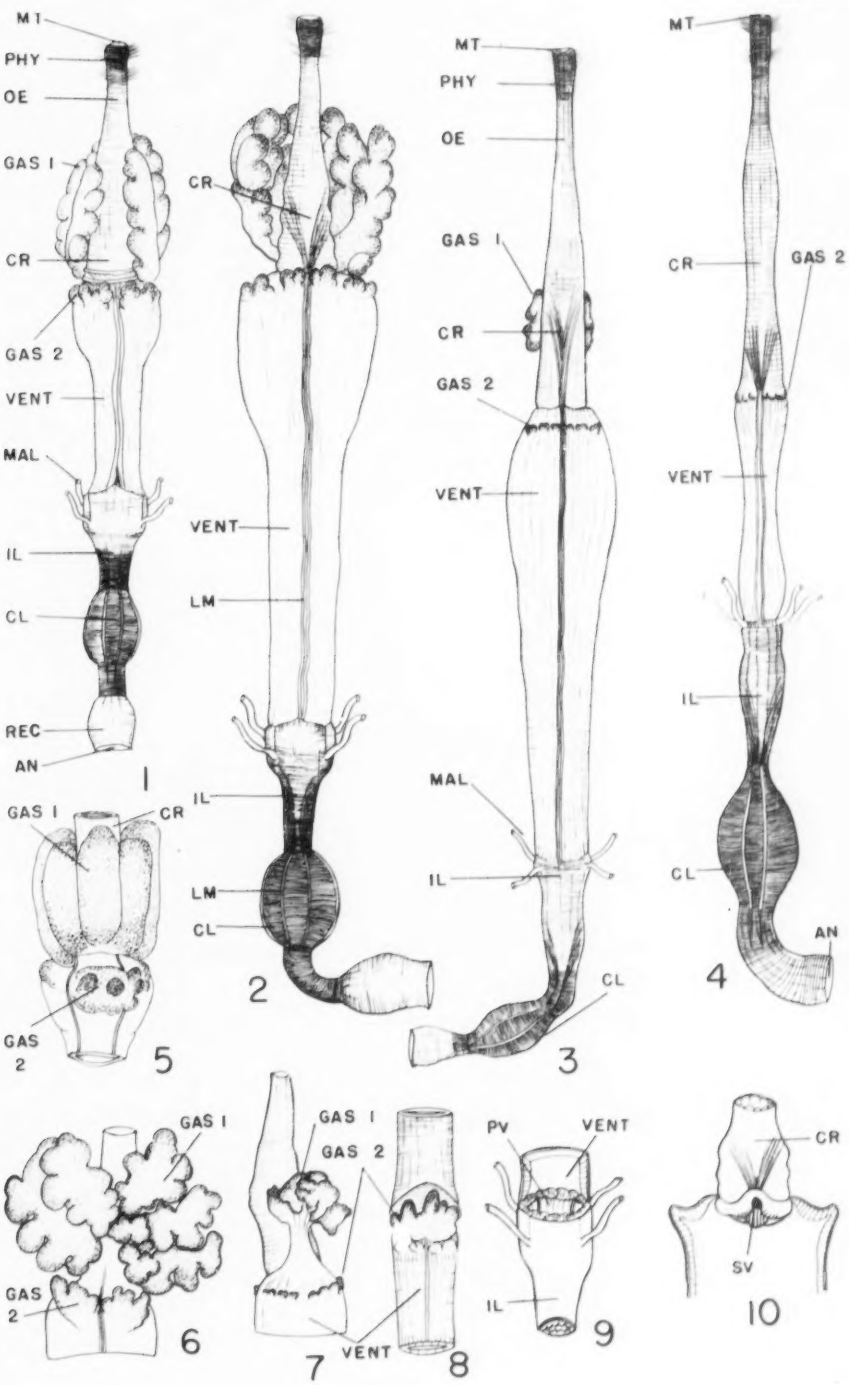
Histologically, the ventriculus of all insect larvae has similar structures. In consists of a layer of outer longitudinal muscles surrounding less developed, isolated circular muscle strands. Lining the inner surface of circular muscles is a layer of well developed epithelial cells (fig. 29, 43, 44, 45, 50). These cells are irregular, more or less columnar or cuboidal in shape. The striated border can be seen at the distal end of the epithelial cells, under the oil immersion lens. In some sections, however, this striated border is indistinct especially in the gut of *Lichnanthe* larvae. The epithelial cells usually contain very large and distinct nuclei.

Groups of small replacement cells with three or four very distinct nuclei in each group, have

EXPLANATION OF PLATE I

The alimentary canal of *Pleocoma crinita* Linsley. FIG. 1. The alimentary canal of the first instar. FIG. 2. The alimentary canal of the fourth instar. FIG. 3. The alimentary canal of the late instar with reduced gastric caeca. FIG. 4. The alimentary canal of the late instar without gastric caeca. FIG. 5. Two sets of gastric caeca of the first instar. FIG. 6. Two sets of gastric caeca of the fourth instar. FIG. 7. Two sets of gastric caeca of the eighth to eleventh instars. FIG. 8. Two sets of gastric caeca of the late instar. FIG. 9. View of pyloric valve. FIG. 10. View of stomodeal valve. AN, anus; CL, colon; CR, crop; GAS, gastric caeca; IL, ileum; LM, longitudinal muscles; MAL, malpighian tubule; MT, mouth; OE, oesophagus; PHY, pharynx; PV, pyloric valve; REC, rectum; SV, stomodeal valve; VENT, ventriculus.

PLATE I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been found resting on the basement membrane at the base of the epithelium. Fletcher (1930, p. 113) stated that secretion of the mid-gut of adult *Phyllophaga gracilis* Burm. seemed to be of the holocrine type, where the entire contents of the cell burst out into the lumen of the intestine, after which the cell is replaced by new cells. Jones (1940, p. 97) also found this condition in the mid-gut of adult *Diplotaxis*. The type of secretion is presumed to be holocrine in all the genera which were studied here. The formation of new cells is accomplished by the division of cells within round, cellular aggregations of nidi, most of which have dropped below the epithelium while some lie among the strands of circular muscles (fig. 29). These replacement cells are nearly equal in size, and do not form the usual bud-like nidus. Nidi are not usually found among the cells of the epithelial layer in the mid-gut of *Pleocoma*, *Lichnanthe* and *Dichelonyx* larvae, but, on the other hand, the formation of them is common in *Polyphylla*.

A very thin, structureless, peritrophic membrane may be found surrounding the food within the mid-gut. Waterhouse and Day (1953, p. 285) stated that two types of peritrophic membranes occur; a single, uniform continuous structure and a tube consisting of a series of concentric lamellae. A peritrophic membrane of the first type is found in the mid-gut of *Polyphylla* which is a very thick and distinct membrane. This is formed by a group of epithelial cells of the mid-gut located around the anterior end (fig. 44). The peritrophic membranes of *Pleocoma* and *Dichelonyx* are very thin and evidently of the second type because they are formed by the epithelial cells throughout the mid-gut (figs. 43, 45). A peritrophic membrane is not found in the canal of *Lichnanthe* larvae.

Gastric caeca.—The diverticula of the mid-gut (gastric caeca) consist of blind pouches varying in number and length and they may be found on different parts of the gut. Rapp, (1947, pp. 145–147) in examining the internal anatomy of several specimens of scarabaeid larvae, found that the gastric caeca varied in number and shape with the species which he observed. According to Rapp, larvae of *Canthon pilularius* (Linn.) (Coprinae) contain no trace of gastric caeca. In *Phyllophaga* (Melolonthinae) he found one set of two gastric caeca at the anterior end of the ventriculus. Two genera in the subfamily Melolonthinae, *Dichelonyx* and *Phyllophaga*, were similar both in number and location of the gastric caeca (fig. 13). In *Polyphylla* the number and location are quite different; it contains three sets of gastric caeca (fig. 12). The first set consists of small lobes located around the anterior end of the ventriculus. In the second set are eight small blind pouches surrounding the middle part of the mid-gut. In the third set are two groups of large lobes located at the dorsal and ventral portion of the posterior end of the mid-gut. Two sets of gastric caeca were found in *Pleocoma* (Pleocominae) and *Lichnanthe* (Glaphyrinae), and two sets have been reported for the larvae of *Pelidnota* (Rutelinae) and *Dynastes* and *Ligyrodes* (Dynastinae) (Rapp, 1947). In *Pleocoma* larvae, both sets are located at the anterior end of the mid-gut (fig. 2). The first set is very large and branches into five lobes covering the underside of the crop. The second set consists of small blind pouches encircling the ventriculus just posterior to the first set. In *Lichnanthe* the first and second sets are located at about one-fourth the distance from the anterior end and posterior end of the mid-gut respectively (fig. 11). Each set consists of eight small blind pouches of the same size encircling the gut. Three and four sets of gastric caeca were found by Rapp (1947, (Cetoniinae), respectively. These are located in a different position on the ventriculus. According to Rapp, the gastric caeca in scarabaeid larvae, when present, may number up to at least four sets and may be located on any portion of the ventriculus.

The structures of the gastic caeca do not differ from those of the ventriculus except that the number of epithelial cells seems to increase tremendously at times (fig. 30). The peritrophic membrane extended from the ventriculus into gastric caeca. However, there was no evidence of the epithelial cells of the gastric caeca secreting this membrane.

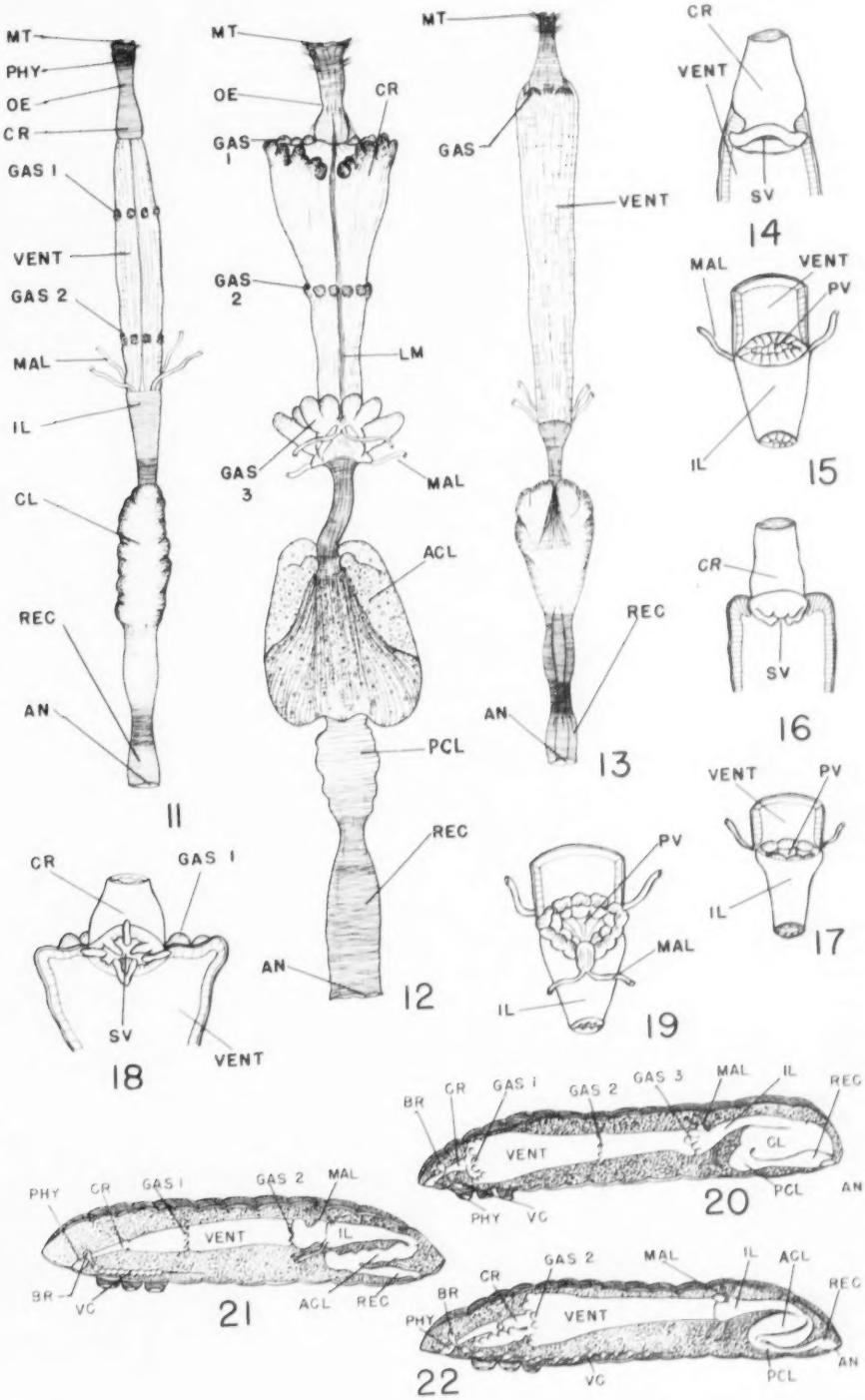
Hind-gut.—The hind-gut is marked anteriorly by the pyloric valve and posteriorly by the anus. It is a rather long tube when compared with the other parts of the gut. In *Pleocoma*, *Lichnanthe*, *Polyphylla*, and *Dichelonyx*, the hind-gut is about 25%, 60%, 50%, and slightly less than 50% of the total length, respectively. Usually it is located in the last three abdominal segments. Generally the hind-gut is differentiated into an ileum, a colon and a rectum.

The hind-gut of scarabaeid larvae studied shows a similarity in the arrangement of tissue layers. The external circular muscles surround

EXPLANATION OF PLATE II

FIG. 11. *Lichnanthe* larva, alimentary canal. FIG. 12. *Polyphylla* larva, alimentary canal. FIG. 13. *Dichelonyx* larva, alimentary canal. FIG. 14. *Lichnanthe* larva, stomodeal valve. FIG. 15. *Lichnanthe* larva, pyloric valve. FIG. 16. *Dichelonyx* larva, stomodeal valve. FIG. 17. *Dichelonyx* larva, pyloric valve. FIG. 18. *Polyphylla* larva, stomodeal valve. FIG. 19. *Polyphylla* larva, pyloric valve. FIG. 20. *Polyphylla* larva, the alimentary canal and nerve cord, diagrammatic. FIG. 21. *Lichnanthe* larva, the alimentary canal and nerve cord, diagrammatic. FIG. 22. *Pleocoma* larva, the alimentary canal and nerve cord, diagrammatic. ACL, anterior colon; BR, brain; PCL, posterior colon; VC, ventral nerve cord. Other abbreviations as on Plate I.

PLATE 2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the isolated bundles of longitudinal muscles throughout the gut. Epithelial or hypodermal cells, which occur as a layer in the innerside of longitudinal muscles, are usually cuboidal in shape, and contain very large nuclei. The basement membrane is well developed. The noncellular intima originates from the point of division between the mid- and hind-gut and is homologous with the cuticula of the body wall at the anus. Numerous isolated strands of longitudinal muscle tissue are scattered along the outside of the external circular muscle layer. These muscle strands are quite prominent at the anterior ileum, some parts of the colon and the rectum.

Ileum.—the initial portion of the hind-gut is the ileum. It is a short and fairly narrow tube which gradually tapers to the posterior end. The ileum is marked anteriorly on the innerside by the pyloric valve. The valve is formed by a group of high columnar epithelial cells of the hind-gut (figs. 46, 47, 48, 51). These cells rise abruptly to form a ring of greatly elongated cells which extends into the lumen of the gut. The inner margin of the valve is covered with a thick intima. Numerous thick folds of the ental surface of the gut can be observed in this region (fig. 33). No teeth or other special structures have been observed. The circular muscles which bound the outside of the ileum are well developed and are thicker toward the posterior end. The longitudinal muscles on the other hand are not well developed. They are present as isolated strands and in the cases of *Dichelonyx* and *Pleocoma* these muscles are found inserted between the folds of epithelial layer of the pyloric valve (figs. 46, 48). The walls formed by hypodermal cells of the ileum along with the thick intima are usually thrown into the longitudinal folds throughout the lumen. These folds cause the lumen to be narrower at the posterior end.

Malpighian tubules.—The malpighian tubules typically arise from the anterior end of the hind-gut according to Snodgrass (1935, p. 378). In the case of scarabaeid larvae this does not hold true in all genera. In the adult *Phanaeus* Becton (1930, p. 316) found two malpighian tubules attached anteriorly to the pyloric valve and two posteriorly to this valve. The writer found this also true for *Polyphylla* larvae. Two of the tubules are located separately on the dorsolateral side just posterior to the proctodeal valve, while the other two are located closely together and have the same opening on the ventral side of the gut just anterior to the valve (figs. 12, 19). In the case of *Dichelonyx* larvae, four malpighian tubules are found rising separately on the gut, just anterior to the pyloric valve. In contrast, with *Dichelonyx*, *Lichnanthe* larvae the four malpighian tubules are attached just posterior to the valve. *Pleocoma* larvae, on the other hand, have four malpighian tubules opening separately at about one-fourth of the total distance from the anterior end of the ileum (fig. 2). The location of the malpighian tubules changes in the different stages of *Pleocoma* larvae and of the adults. Four malpighian tubules are also found in *Phanaeus* (Becton, 1930, p. 316), *Diplotaxis* (Jones, 1940, p. 95), *Popillia* (Swingle, 1950, p. 190), and *Oryctes* (Gressitt, 1953, p. 27).

Each malpighian tubule is a single, unbranched tube and the same diameter throughout. The malpighian tubules of the larvae studied here are of the type described by Roeder (1953, p. 391) as being found principally among the Coleoptera and Lepidoptera. The tubes extend anteriorly and turn back posteriorly. The distal ends of the tubes are attached to the rectum. Actually, they lie on the surface of the rectum and are held in place by an enveloping membrane. In all the larvae studied, the tip of all tubules was attached at the same point on the rectum.

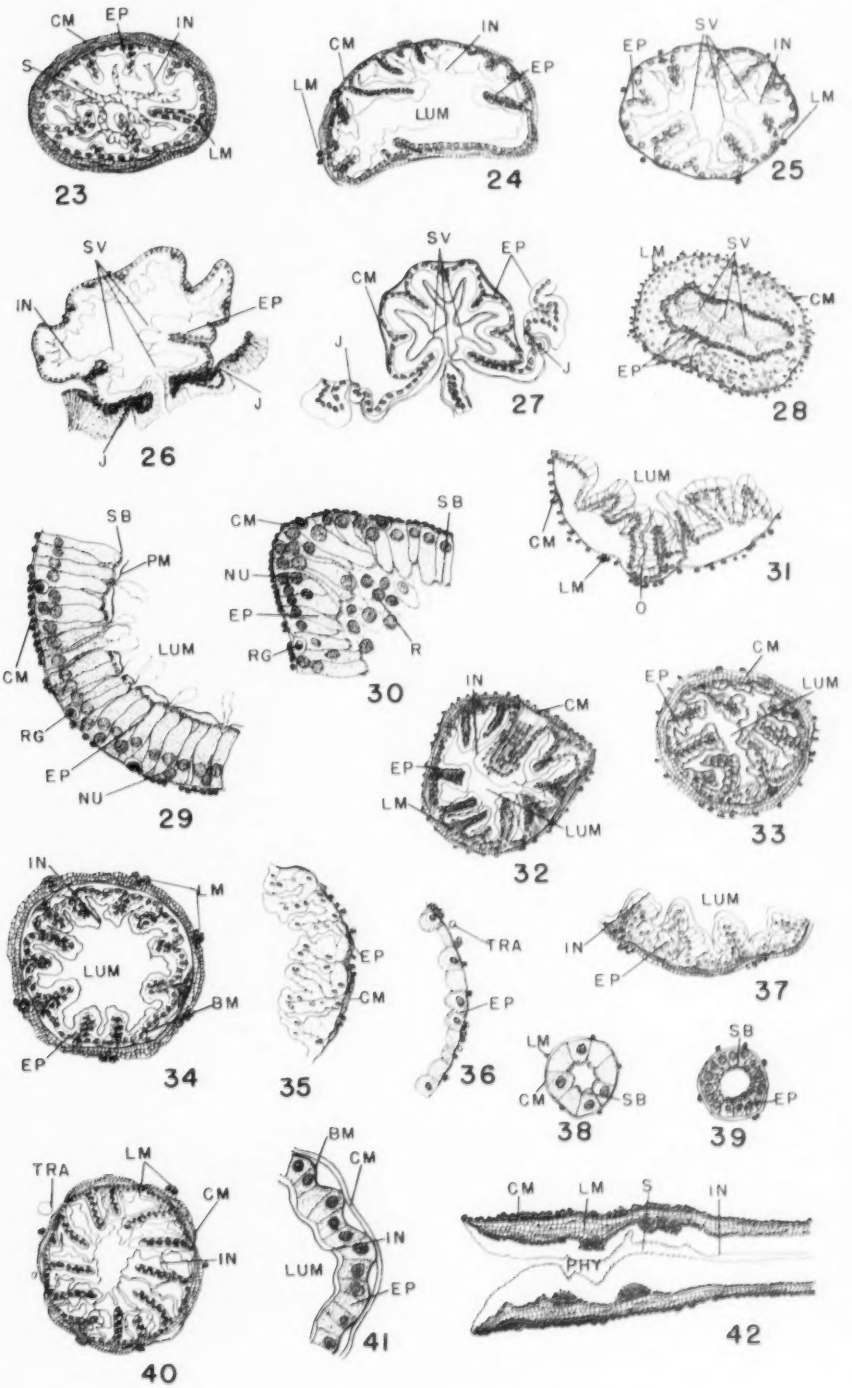
The malpighian tubules consist of a layer of columnar epithelial cells surrounded by thin circular muscles (figs. 38, 39). These cells contain quite large and distinct nuclei. The number of cells in cross section vary within the tube and in different insects. In *Pleocoma*, *Lichnanthe*, *Dichelonyx* and *Polyphylla*, 7 to 15, 3 to 6, 4 to 9, and 6 to 8 cells were found respectively. The striated border can be seen at the distal end of the epithelial cells. However, in some sections this striated border is indistinct. The intima layer is absent. On the outside, the malpighian

EXPLANATION OF PLATE III

FIG. 23. *Polyphylla* larva, cross section of pharynx. FIG. 24. *Pleocoma* larva, cross section of crop. FIG. 25. *Dichelonyx* larva, cross section through region of stomodeal valve. FIG. 26. *Pleocoma* larva, cross section through region of stomodeal valve. FIG. 27, *Polyphylla* larva, cross section through region of stomodeal valve. FIG. 28. *Lichnanthe* larva, cross section through region of stomodeal valve. FIG. 29. *Polyphylla* larva, cross section of a portion of ventriculus. FIG. 30. *Dichelonyx* larva, cross section of a portion of gastric caeca. FIG. 31. *Polyphylla* larva, cross section of a portion of pyloric valve. FIG. 32. *Lichnanthe* larva, cross section of ileum. FIG. 33. *Pleocoma* larva, cross section of ileum. Fig. 34. *Pleocoma* larva, cross section of anterior colon. FIG. 35. *Lichnanthe* larva, cross section of a portion of anterior colon. FIG. 36. *Polyphylla* larva, cross section of a portion of anterior colon. FIG. 37. *Polyphylla* larva, cross section of a portion of posterior colon. FIG. 38. *Dichelonyx* larva, cross section of malpighian tubule. FIG. 39. *Pleocoma* larva, cross section of malpighian tubule. FIG. 40. *Pleocoma* larva, cross section of posterior colon. FIG. 41. *Polyphylla* larva, cross section of a portion of rectum. FIG. 42. *Pleocoma* larva, longitudinal section of larval pharynx.

BM, basement membrane; CM, circular muscles; EP, epithelial cells; IN, intima; J, junction of fore- and mid-gut; LM, longitudinal muscles; LUM, lumen; NU, nucleus; O, malpighian tubule orifice; PHY, pharynx; PM, peritrophic membrane; RG, regenerative cells; S, spines; SB, striated border; SV, stomodeal valve; TRA, trachea.

PLATE 3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tubules are bounded by a narrow layer of connective tissue.

Colon.—The colon of scarabaeid larvae constitutes the most outstanding part of the alimentary canal. It is usually divided into an anterior and a posterior colon, which are folded under the ileum. The colon may differ in shape and structure in different genera. Generally the anterior colon is a very large thin-walled sac. This is found in *Polyphylla* (fig. 12), *Dichelonyx* (fig. 13), *Oryctes* (Gressitt, 1953, p. 28) and *Popillia* (Swingle, 1950, p. 190). Histologically the epithelial cells are of a broad cuboidal shape, and scattered on a very distinct basement membrane (fig. 36). The outer circular muscle layer is thin but well developed. The longitudinal muscles are not well developed and only an occasional strand is found. Some strands of longitudinal muscles extend across the upper surface of the gut from the ileum and posterior colon. These muscles probably aid in the contraction of the colon. The intima is very thin and distinct.

The anterior colon of *Lichnanthe* larva is different in shape and structure from the above. It forms a rather long tube with irregular transverse folds appearing inside (fig. 11). The folds are caused by the layer of epithelial cells pulling inward toward the lumen. Other structures such as circular muscles, longitudinal muscles and intima do not exhibit any differences from other scarabaeid larvae.

The anterior colon of *Pleocoma* is nearly spherical in shape (fig. 2). It differs from other genera in that the external circular muscles are very thick, with six distinct strands of longitudinal muscles forming a layer with a distinct basement membrane. This layer forms numerous folds inside the gut (fig. 34).

The posterior colon of *Dichelonyx* and *Polyphylla* is a narrow sac. It differs from the anterior colon in that the circular muscles are very thick in this region. Numerous folds are found in the epithelial layer and the intima is very thick and distinct. In the case of *Lichnanthe* and *Pleocoma*, the posterior colon is a very narrow tube. Thus, the invagination of the epithelial layer and the thick intima causes the lumen to be very narrow. The circular muscles are much thicker than those of the anterior part. The longitudinal muscles are not well developed in *Lichnanthe* but in *Pleocoma* longitudinal muscles are found inserted between the folds of the epithelial layer. The basement membrane is very distinct in all genera.

Rectum.—Externally the rectum is a narrow sac resulting from the dilation of the posterior end of the alimentary canal (figs. 2, 11, 12, 13). The circular muscles are well developed, but are weak in some parts, especially in the middle part (fig. 41). The epithelial layer is slightly folded. It consists of regular cuboidal cells with very distinct nuclei. The longitudinal muscles are few and inconspicuous. A small number of isolated groups of muscles comparable to those in the posterior colon are present. The chitinous intima is very thick. The basement membrane is very distinct. Generally, the structures of the rectum of the larvae which were studied did not differ.

NERVOUS SYSTEM OF SCARABAEIDAE

Changes During Larval Development.—Several instars of *Pleocoma* were observed (see Table I). The nervous system consists of these divisions: the brain; the stomodeal nervous system which is composed of a frontal ganglion, a hypocerebral ganglion, a pair of oesophageal ganglia; a suboesophageal ganglion; three thoracic ganglia and eight abdominal ganglia. No major changes are found during the development of the instars studied, the nerves and ganglia remaining the same in number throughout the larval stages. Only minor changes, such as shortening of connectives and an anterior movement of posterior ganglia can be observed. The abdominal ganglia became more and more elliptical and somewhat reduced in size as the larvae grow older.

The shortening of the nerve cord during the growth of the larva brings about a change in position of the ganglia. The brain, the suboesophageal, thoracic and the first abdominal ganglia remain in the same position in all larval stages, while the position of the abdominal ganglia changes in different instars. Thus, in the 2- and 10-day old first instars, ganglia 2 to 6 are located in abdominal segments 1 to 5 respectively, while ganglia 7 and 8 are found in the abdominal segment 6.

In the fourth instar, ganglia 2 to 7 are located in segments 1 to 5 and only the last one remains in the segment 6 (fig. 52).

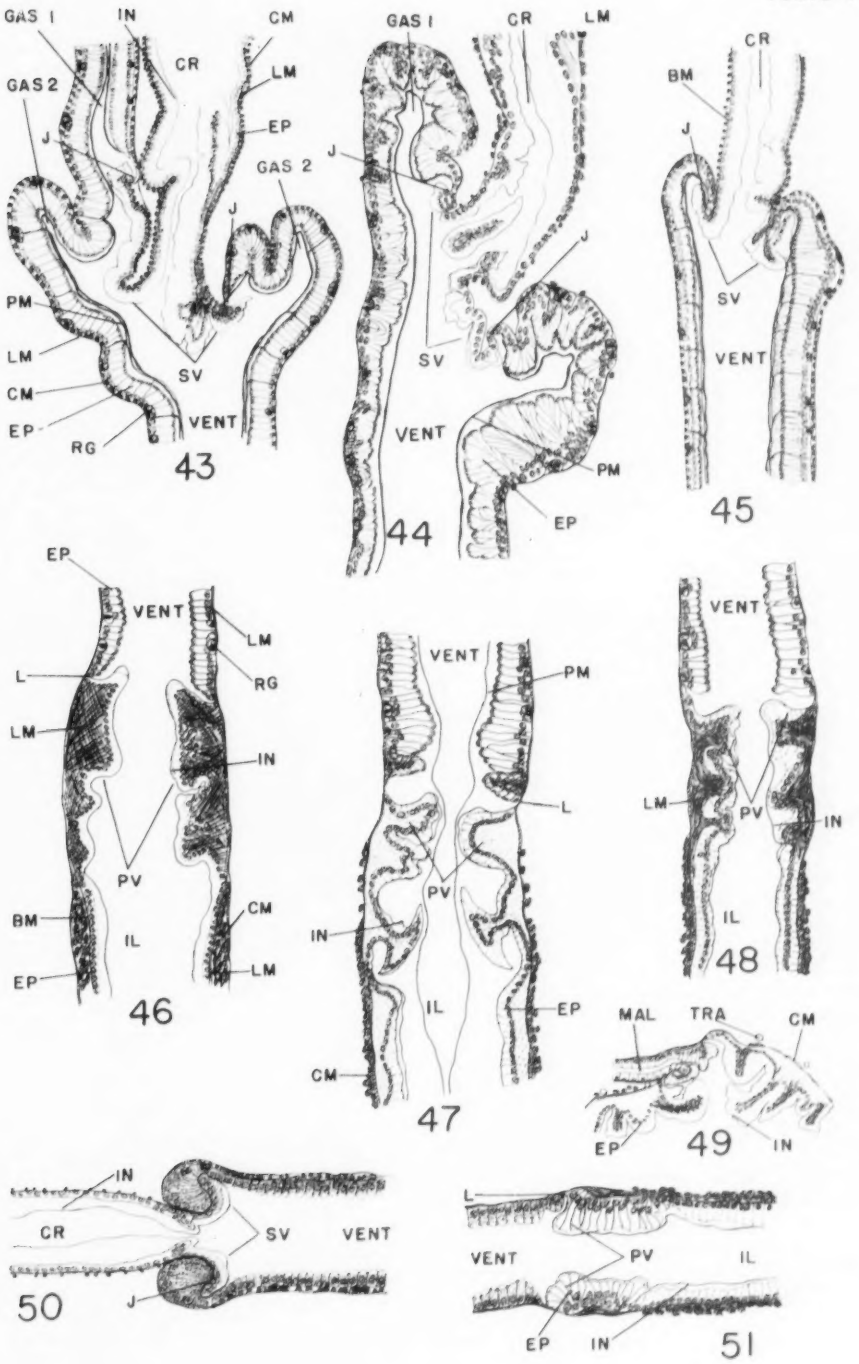
Beyond the fourth instar, only a very slight change in position of the nervous system occurred. Ganglia 2 and 3 are in the first abdominal segment, ganglia 4 to 6 lie in abdominal segments 2 to 4, and the last two ganglia remain in the segment 5 (fig. 53).

In both male and female adults, the first

EXPLANATION OF PLATE IV

FIG. 43. *Pleocoma*, longitudinal section of larval stomodeal valve. FIG. 44. *Polyphylla*, longitudinal section of larval stomodeal valve. FIG. 45. *Dichelonyx*, longitudinal section of larval stomodeal valve. FIG. 46. *Pleocoma* larva, longitudinal section of the mid- and hind-gut junction. FIG. 47. *Polyphylla* larva, longitudinal section of the mid- and hind-gut junction. FIG. 48. *Dichelonyx* larva, longitudinal section of the mid- and hind-gut junction. FIG. 49. *Lichnanthe* larva, section of proctodeal pyloric valve. FIG. 50. *Lichnanthe*, longitudinal section of larval stomodeal valve. FIG. 51. *Lichnanthe*, longitudinal section of the mid- and hind-gut junction of larva. L, junction of mid- and hind gut. Other abbreviations as on previous plates.

PLATE 4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abdominal ganglion becomes fused with the third and second thoracic ganglia and moves anteriorly into the first thoracic segment. The chain of the remaining five abdominal ganglia begins in the mesothorax and ends in the first abdominal segment. Since the last abdominal ganglion of the adults contains five pairs of lateral nerves rather than the three found in larvae, this ganglion is probably formed by the fusion of the last three abdominal ganglia of larval stages (fig. 54).

No changes of the nervous system were found in the second and third instars of *Lichnanthe*, *Polyphylla* and *Dichelonyx* larvae.

Comparisons of Nervous System of Several Genera.—The most important differences in the nervous system are exhibited in the ventral cord of many species of adult Coleoptera. Brandt (1879), after studying many species of beetles, both adults and larvae, concluded that the most generalized type of nervous system, in addition to the supra- and infra-oesophageal centers, consists of three thoracic ganglia and eight abdominal ganglia. For example, in *Dictyopterus* there is a reduction in the number of abdominal ganglia, accompanied by a similar specialization of the thoracic centers. Such reduction may be traced through a number of genera of Coleoptera. Thus, there are two, three, four, five, six, and seven abdominal ganglia in *Coccinella*, *Cassida*, *Donacia*, *Silpha*, *Cicindela* and *Lampyris* respectively. Also in a number of other Coleoptera and particularly in the family Scarabaeidae, the second and third thoracic ganglia are closely united together as in *Melolontha*, *Phyllopertha*, *Cetonia*. The center thus formed also includes the fused ganglia of the abdominal chain. *Serica* and *Rhizotrogus* are recognized as the most specialized genera. In these genera all the thoracic and the abdominal ganglia unite to form a single complex nervous center in the thorax. In *Rhizotrogus*, the sub-oesophageal ganglion also coalesces.

Larvae of the various subfamilies of Scarabaeidae which the writer has observed also show various types of a nervous system. *Pleocoma* represents the most primitive type, *Polyphylla* the highest type, while *Dichelonyx* and *Lichnanthe* represent intermediate types.

Brain.—The brain of scarabaeid larvae is composed of a pair of large simple lobes located in the cephalic region. The compact pear-shaped white masses of the brain show no distinct divisions. The location of divisions can be determined only by the location of the principal nerves. In the four genera studied the nervus opticus, nervus antennalis, nervus lateralis, frontal ganglion connective and labral nerve are present.

Stomodeal Nervous System.—The stomodeal nervous system consists of a frontal ganglion, a hypocerebral ganglion, a pair of oesophageal ganglia and a pair of stomachic ganglia. These ganglia are well developed in *Pleocoma* and *Dichelonyx* larvae. The hypocerebral ganglion is degenerated in the nervous system of *Polyphylla* and all the ganglia, except the frontal ganglia, are undeveloped in *Lichnanthe* larva (figs. 53, 56, 57, 59).

Sub-oesophageal Ganglion.—The sub-oesophageal ganglion is large and located in the cephalic region. It is joined with the brain by a pair of circumesophageal connectives. The peripheral nerves of this ganglion innervate the mandibles, the hypopharynx, the maxillae, and the labium. The shape of the ganglion is usually flat and nearly circular. In the more specialized forms, as stated above, this ganglion is fused with the thoracic ganglion. Thus, *Polyphylla* is an example of this type (fig. 56). In the other three genera, this ganglion remains separate and is joined with other ganglia by a pair of connectives.

Thoracic Ganglia.—In the most generalized form, the thoracic ganglia are three in number and are joined together with other ganglia by a pair of connectives. They are usually nearly spherical in shape and are a little larger than the abdominal ganglia. The shortening of the connective nerves causes the ganglia to move anteriorly which can be traced in the genera which were studied. In *Pleocoma* larvae the thoracic ganglia have rather long connectives and are located in thoracic segments 1, 2, 3 respectively (fig. 53). In *Lichnanthe* and *Dichelonyx* larvae the thoracic ganglia have rather short connectives and are found in thoracic segment 1 and 2 (figs. 57, 59). In *Polyphylla* on the other hand, the connectives are absent. Here the thoracic ganglia are fused with the sub-oesophageal ganglion. The resulting ganglion is located between the cephalic region and the first thoracic segment (fig. 56).

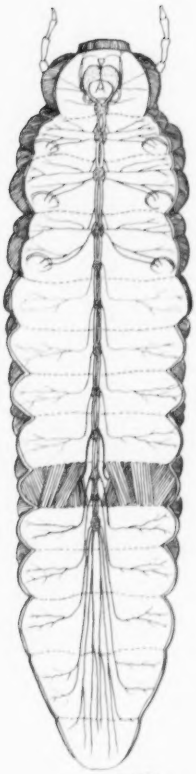
Abdominal Ganglia.—In the more primitive species there are eight abdominal ganglia which are equal in size except the last one which is slightly larger. Similar to the thoracic ganglia, these ganglia have rather long connectives and form the long chain of the ventral nerve cord in the more generalized form. Thus, in *Pleocoma* larva, eight abdominal ganglia are joined together by a long pair of connectives and are located in the region between the third thoracic segment and

EXPLANATION OF PLATE V

Ventral nervous system and brain of scarabaeids. FIG. 52. *Pleocoma*, fourth larval stage. FIG. 53. *Pleocoma*, late instar. FIG. 54. *Pleocoma*, adult stage. FIG. 55. *Polyphylla*, larval stage. FIG. 56. *Polyphylla*, larval stage, showing the brain, the ventral and stomodeal nervous system. FIG. 57. *Lichnanthe*, larval stage. FIG. 58. *Geotrupes blackburni*, larval stage. FIG. 59. *Dichelonyx*, larval stage.

A, abdominal segment; ATN, nervus antennalis; BR, brain; FG, frontal ganglion; H, head; HG, hypocerebral ganglion; LN, nervus lateralis; OG, oesophageal ganglion; ON, nervus opticus; SC, sub-oesophageal commissure; SO, sub-oesophageal ganglion; T, thoracic segment, I, prothoracic ganglion; II, mesothoracic ganglion; III, metathoracic ganglion; 1–8 abdominal ganglia.

PLATE 8



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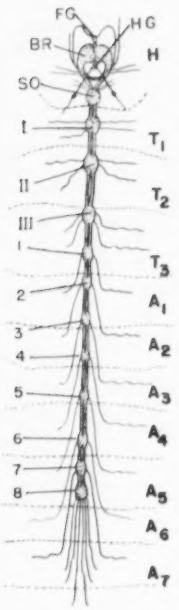

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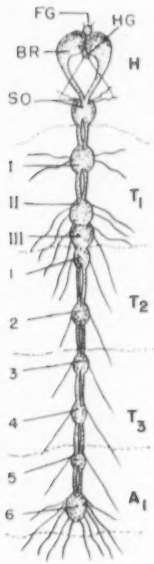

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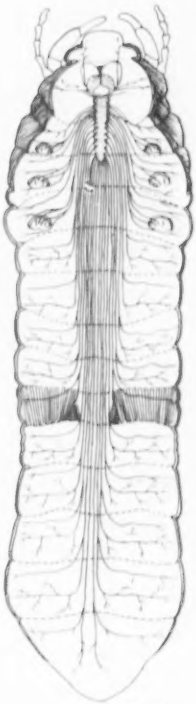

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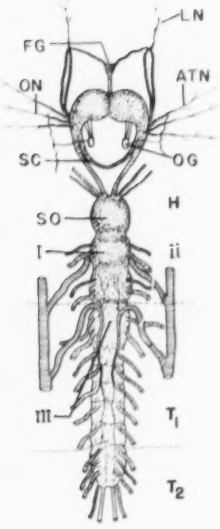

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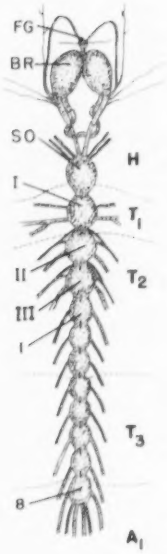

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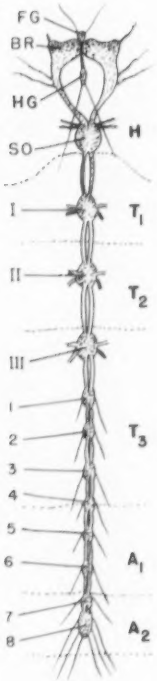

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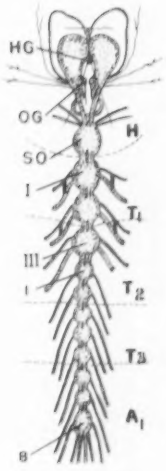

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the fifth abdominal segment (fig. 53). On the other hand, the eight abdominal ganglia of *Lichnanthe*, as well as of *Dichelonyx* are joined by a very short pair of connectives. In *Lichnanthe* larva, the first three ganglia are located in the second thoracic segment. The third thoracic segment contains four ganglia and only one ganglion is in the first abdominal segment (fig. 57). In *Dichelonyx* larva the first two ganglia are located in the second, and the next three are in the third thoracic segment. The last three ganglia remain in the first abdominal segment (fig. 59). The abdominal ganglionic chain of *Polyphylla* shows a very specialized form. All the ganglia are fused into a short narrow mass forming a ganglion located in the first thoracic segment. The slight constrictions of this ganglion and the peripheral nerves show that probably six ganglia of the abdominal ganglionic chain have fused together (fig. 56). The last ganglion probably results from the fusion of three ganglia during the early development stage.

Peripheral Nerves.—In studying the peripheral nerves of the ventral nerve cord of several genera, it is found that each thoracic ganglion contains two pairs of lateral nerves and each abdominal ganglion except the last one, has one pair. Three pairs of peripheral nerves are present on the last abdominal ganglion. These nerves usually arise from the lateral side of the ganglia except in the *Polyphylla* larva where it is found on the dorso-lateral sides. Each thoracic or abdominal segment is innervated by peripheral nerves from the respective thoracic or abdominal ganglia. Only the last abdominal ganglion is found to supply the last three abdominal segments (fig. 52).

It should be noted here that the peripheral nerves supply the same segments in which they were originally located, although there has been a slight shortening of the nerve cord, a condensation of ganglia, and a loss of connectives as found in *Pleocoma* and *Polyphylla* larvae (figs. 52, 55).

Nervous System of Other Scarabaeid Larvae.—Observations were made on the nervous system of some other scarabaeid larvae. In *Geotrupes blackburni* (Fab.) (Geotrupinae), the brain is rather peculiar in shape and the sub-oesophageal ganglion is very distinctly located in the cephalic region. The ventral nerve ganglia are joined together by a long pair of connectives. A thoracic ganglion is located in each of the three thoracic segments. The eight abdominal ganglia are found beginning in the third thoracic and ending in the second adbominal segment. The seventh and eighth abdominal ganglia are nearly fused together (fig. 58). In the genus *Trox* of the subfamily Troginae, the writer has found that the central nerve cord resembles that of *Pleocoma*, except in *Trox* the number of abdominal ganglia are reduced to six. In *Pelidnota punctata* (L.) (Rutelinae), the central nerve cord is very specialized and similar to *Polyphylla* larva. Other genera which were observed to be of this type are *Xyloryctes* (Dynastinae) and *Osmoderma* (Cetoniinae).

Oryctes rhinoceros (Dynastinae) according to Gressitt (1953, p. 29) probably has the most specialized nervous system of the Scarabaeidae. The larva has only a pro- and a mesothoracic ganglion. The mesothoracic ganglion is partially fused with the common metathoracic abdominal ganglion, so that this fused ganglion appears to have 12 pairs of peripheral nerves in the third larval instar. In the adult stage all of these ganglia fuse with the sub-oesophageal ganglion to become one large lobe.

Also it should be mentioned that in the genus *Ceruchus* (Lucanidae) the ventral nerve cord is of the same type as in *Pleocoma*. This type is also found in *Passalus* larvae (Passalidae) which were studied by Cody and Gray (1938, p. 508). In adult *Passalus*, the central nerve cord is very much different from the larval cord. The meso- and metathoracic ganglia which are fused with the abdominal ganglia, are located in the second thoracic segment while the prothoracic ganglion is separated in the first segment.

DISCUSSION

Beginning with the most primitive and placing the most specialized family last, Leng (1920, pp. 248–265) arranged the Scarabaeoidea phylogenetically as follows: Scarabaeidae, Trogidae, Lucanidae and Passalidae.

The family Scarabaeidae contains many subfamilies. Beginning with the most generalized the important subfamilies have the following phylogenetic arrangement according to Leng: Coprinae, Geotrupinae, Pleocominae, Glaphyrinae, Troginae, Melolonthinae, Rutelinae, Dynastinae, and Cetoniinae.

These phylogenetic concepts have been further tested by the writer by studying the digestive tract and the nervous system of the larva of one species from each of the following subfamilies: Pleocominae, Glaphyrinae, Melolonthinae.

Recently Maxwell (1955, pp. 102–115) has shown that the salivary glands and the malpighian tubules have considerable phylogenetic significance in the sawfly larvae. Also, she believes these characters to be of phylogenetic significance, but not of the same magnitude: variation in gut proportions, size and structure of the crop, differences in cuticular covering, variations in the epithelial lining of the gut, presence and absence of spines in the gut, number of the gastric caeca, and the structure of the individual malpighian tubules. Studies of the present writer indicate that both adaptive and phylogenetic features are to be found in the alimentary canal of the Scarabaeidae.

Adaptive Features of Alimentary Canal.—The length of the gut in scarabaeid insects varies from one genus to another. Many workers consider

the length of the gut as being related to the adaptation for feeding. Dung feeding insects such as *Canthon*, usually have a relatively longer digestive tract than fresh-tissue feeding insects. Also there is some evidence that the length of the canal, although differences are not great, might be correlated with the development of gastric caeca. The longer the alimentary canal, the more the gastric caeca are reduced and degenerated. This reaches the ultimate in *Canthon* dung feeding larvae which have no gastric caeca and the alimentary canal is twice as long as the body. The dead leaf feeding *Lichnanthe* larvae have two sets of undeveloped gastric caeca and the canal is 1.8 times the body's length. *Dichelonyx* and *Pleocoma* larvae feed on live roots. The former larvae have one set of well developed gastric caeca and a gut that is 1.5 times the length of the body, while *Pleocoma* has two well developed caeca and the canal is 1.3 times as long as the body. Thus, the feeding habits of scarabaeid larvae seem to be correlated to some degree with the development of the gut and gastric caeca.

Changes in the number of gastric caeca and changes in the length of the alimentary canal during the development of larval stages was studied in *Pleocoma*. The length of the fore-, mid-, and hind-gut and the development of gastric caeca are different between the first and the fourth instar and between the eighth and ninth instar. The changes are correlated with the tremendous increase of the epithelial cells in the mid-gut and in the gastric caeca from the first instar to fourth and from the fourth to eighth instars. These early instars are considered by the writer to be the active feeding stage and at this time active secretion of digestive juices would be needed. The reduction of these cells is followed by the shortening of the mid-gut and the degeneration of the gastric caeca, which occurs about the ninth instar. The author suggests that the degeneration of the gastric caeca indicates either a cessation of feeding or indicates a change in feeding habits. These changes do not occur in the other genera studied.

Morphological and Phylogenetic Considerations of Alimentary Canal.—Scarabaeid larvae possess a very short fore-gut and a rather long mid-gut. The hind-gut may be short or long depending on the species. Also, these differences are present: presence or absence of spines in the fore-gut; changes in the shape of the stomodeal valve; the presence or absence of the peritrophic membrane; the number, shape, and location of the gastric caeca; the structure and location of the malpighian tubules; the changes in form and structure of the colon; and the musculature of some parts of the gut.

The intima of the pharynx of *Pleocoma*, and *Dichelonyx* has a vestiture of retrose spines but such spines are absent in *Polyphylla* and *Lichnanthe*. These spines are also found in adults of many genera, such as *Phanaeus*, *Phyllophaga*, *Diplotaxis*, *Popillia*, etc. The peritrophic membrane of the mid-gut of *Polyphylla* forms a single, uniform continuous structure while in *Pleocoma* and *Dichelonyx* the peritrophic membrane forms a series of concentric lamellae. The membrane is absent in *Lichnanthe*. The shape of the stomodeal valves depends upon the shape of the lobes functioning as valves. In *Pleocoma* there are three large lobes, in *Polyphylla* six large lobes, in *Dichelonyx* numerous lobes. There is a thick ring in *Lichnanthe* (figs. 10, 14, 16, 18). The histological structure is similar in these genera. In the musculature there were such differences as the insertion of longitudinal muscles on the proctodeal valve of *Pleocoma* and *Dichelonyx*, while the muscles were absent in *Polyphylla* and *Lichnanthe*. These differences apparently have no significance phylogenetically, since they may occur in any subfamily.

The number of malpighian tubules in the larvae of many subfamilies of Scarabaeidae is the same. Even though their structures are the same, the location of the tubules differs from one subfamily to the other. The malpighian tubules occur on any portion of the mid- and hind-gut. They were found on the middle and posterior end of the mid-gut in *Osmoderma* (Rapp, 1947, p. 145) and *Dichelonyx* respectively. They may occur both anteriorly and posteriorly on the proctodeal valve as in *Polyphylla* or they may be found only on the posterior portion of the valve as in *Lichnanthe* and *Pleocoma*. Although the histological structures did not differ, yet the number of cells did. In cross sections of *Pleocoma* malpighian tubules, the walls were seven to fifteen epithelial cells thick, while in *Lichnanthe* there are only three to six cells.

The colon shows a diversity in shape and in its structures. In the primitive *Pleocoma* larvae there are two divisions of the colon as in the other genera, but both divisions consist of very thick circular muscles, which are crossed with six isolated strands of longitudinal muscles. A very thick intima is present. In *Lichnanthe* a long broad tube is formed anteriorly and narrow tube posteriorly. The distinct structures are the very weak circular muscles, the indistinct intima of the anterior colon and the very strong circular muscles and the conspicuous intima of the posterior colon. Also in *Dichelonyx*, *Polyphylla*, *Oryctes* (Gressitt, 1953, p. 28), *Popillia* (Snodgrass, 1935, p. 190) and many other genera, the large sac-like anterior colon and the narrow posterior colon are characteristic of the genera. The distinct characters of the anterior colon, like as of *Lichnanthe*, are the weak circular muscles and the inconspicuous intima, but the epithelial cells are broad and irregularly scattered which is different from the epithelial layer in the colon of *Lichnanthe* or *Pleocoma*. The structures of the posterior colon do not differ from the other genera.

Rapp (1947, p. 147) reported that the gastric caeca of scarabaeid larvae differ in number, shape, and location. This is also true for all larvae which were studied by the present writer. Large lobes are found in *Pleocoma*, *Polyphylla* while small blind pouches are found in *Dichelonyx*, *Lichnanthe*. The location of the gastric caeca on the mid-gut differs with genus. Only one set is found in *Dichelonyx*, two sets in *Pleocoma* and *Lichnanthe* and three sets in *Polyphylla* larvae.

While the gastric caeca display adaptations in regard to the type of food eaten by the individual yet apparently a general phylogenetic trend can also be traced. Thus, in the most generalized form there are no gastric caeca, but as the degree of specialization increases, the number of gastric caeca increase. This may be traced through a number of genera. The larvae of *Canthon* (Coprinae), which is considered the most generalized of the *Scarabaeidae*, have no gastric caeca. The larvae of *Phyllophaga* (Melolonthinae), *Pelidnota* (Rutelinae), *Xyloryctes* (Dynastinae), and *Osmoderma* (Cetoniinae), possess one, two, three, and four sets of gastric caeca, respectively.

When the concept that the number of gastric caeca indicates phylogenetic trends is applied, the *Polyphylla* with three sets of gastric caeca is more advanced than *Phyllophaga* (Rapp, 1947, p. 145) and *Dichelonyx* (observed by the writer) which have only two blind pouch-like gastric caeca. At present all three are included in the *Melolonthinae*. As will be discussed later, the nervous system of the larvae of *Polyphylla* is also more advanced than the two latter genera. However, according to Dr. P. O. Ritcher (personal communication) other adult and larval characteristics would indicate that *Polyphylla* is about the same phylogenetic level as other Melolonthinae.

Phylogenetic Consideration of Nervous System.—The value of the nervous system as a phylogenetic indicator is more clear cut than the alimentary canal. Brandt (1879) recognized that the most important differences in the nervous system in Coleoptera larvae are found in the ventral cord. In the most generalized type of a nervous system there are three separate units, the sub-oesophageal ganglion, the three thoracic ganglia and the eight abdominal ganglia. Both the units and their component parts are connected by a pair of connectives. In the more specialized form, the abdominal ganglia become fused so that the number of individual ganglia is reduced. Also the thoracic ganglia are subject to fusion. Likewise the sub-oesophageal ganglion becomes a compact mass. The nervous system of *Pleocoma* larva is an example of the most generalized form in the Scarabaeidae. The nervous system of *Lichnanthe* and *Dichelonyx* is also of the generalized type, but here the connectives are shorter than in *Pleocoma*. On the other hand, in *Polyphylla*, all the ganglia, including the sub-oesophageal ganglion, are fused into a compact mass.

Thus, both the digestive tract and the nervous system of *Polyphylla* larvae are of a specialized type. Furthermore, the *Polyphylla* type of nervous system is found in larvae of the higher specialized subfamilies, as typified by *Pelidnota* (Rutelinae), *Xyloryctes* (Dynastinae) and *Osmoderma* (Cetoniinae).

The number of ganglia which fuse into a compact mass probably can be determined by the number and location of peripheral nerves. In the various scarabaeid larvae which were observed, each thoracic ganglion contains two pairs of peripheral nerves; each abdominal ganglion contains one, except the posterior ganglion contains three. Although there has been a condensation of the ganglia and an anterior migration of the entire cord, the peripheral nerves still supply the same segments in which they were originally located. Also, they still arise from the ganglia and ganlionic mass in their same relative position. However, on studying *Corydalis* larvae, Hammar (1908, p. 120) was unable to count the number of fusing ganglia. He believed that an investigation of the embryological conditions was the only way to settle this problem.

There seem to be a few changes in the nervous system during development of larval stages in scarabaeid insects. There is only a slight anterior movement of posterior ganglia by shortening of the connectives in *Pleocoma* and no changes occur in other genera which were studied here. There is considerable change from the larval to the adult nervous system. In *Pleocoma* the abdominal ganglia are reduced from eight in the larvae to six in the adults. The meso- and metathoracic ganglia are partially fused with the first abdominal ganglion in the adult but not in the larva. *Oryctes cornutus* (Cody and Gray, 1938, p. 513), *Oryctes rhinoceros* (Gressitt, 1953, p. 31) are other examples.

Since the family Passalidae is considered to be more specialized than the Scarabaeidae, an interesting comparison can be made. The nervous system of the larva of *Popilius* (Passalus) as figured by Cody and Gray (1938, p. 519) resembles *Pleocoma* very closely. However, the nervous system of the adult *Popilius* (Passalus) (Cody and Gray, 1938, p. 521) is very specialized and closely resembles *Oryctes* (Gressitt, 1953, p. 31). On the other hand *Oryctes*, contains a very specialized type of nervous system in both larvae and adults. Thus *Passalus* does not exhibit its specialized form of the nervous system in the larval stage but only in the adult stage. The generalized type of a nervous system is also found in the larva of *Ceruchus* (Lucanidae) as observed by the writer.

SUMMARY

The digestive and nervous systems of larvae of

four genera including several instars of *Pleocoma* (Pleocominae), *Lichnanthe* (Glaphyrinae), *Dichelonyx* (Melolonthinae), and *Polyphylla* are described and compared. The results of this investigation can be briefly summarized as follows:

1. There are not many changes in the alimentary canal during the development of larval stages. The only changes are in the length of the mid-gut and in the development of gastric caeca. These changes are accomplished by the development and degeneration of epithelial cells of the mid-gut. In *Pleocoma* larvae these changes occur between the first and the fourth instars and between the eighth and the ninth instars. Such changes do not occur between the second and the third instar of other genera.

2. Only minor changes of the nervous system, such as a slight anterior movement of posterior ganglia and the shortening of connectives, occur during the development of these larval stages.

3. The alimentary canal of the larvae of all the genera studied is not much different both in form and in divisions. These differences in the digestive tract were noted among genera: Spines were present or absent in the fore-gut; the shape of the stomodeal valves differ; peritrophic membrane was present or absent, and its formation differed; the number, form and location of gastric caeca differed; the location and structure of malpighian tubules differed; the form and structures of the colon and the musculature in some particular part of the gut, such as at the region of the pyloric valve, differed.

4. The most important differences in the nervous system in these larvae are exhibited in the ventral nerve cord. In the most generalized system there are three separate units: a suboesophageal ganglion, three thoracic ganglia and eight abdominal ganglia. These are joined together by a long pair of connectives in *Pleocoma* larvae which are considered to be the most generalized of the Scarabaeidae studied. The shortening of the connectives without the fusion of these ganglia in *Lichnanthe* and *Dichelonyx* is considered as a step toward the more specialized forms. The fusion of all ganglia into a long compact mass as found in *Polyphylla*, is considered to be the most specialized type.

5. Because the adaptive structures for feeding influence the shape and form of the digestive tracts of scarabaeid larvae, many structures of the alimentary canal are not of phylogenetic significance. There are however some evidences that the gastric caeca and the ventral nerve cord are important as phylogenetic indices.

6. The gastric caeca and the ventral cord of *Pleocoma*, *Lichnanthe*, *Dichelonyx* are of the generalized type which is in agreement with the phylogenetic concept of authors in arranging subfamilies. On the other hand *Polyphylla* (Melolonthinae) is more highly specialized.

7. More extensive studies are needed for determining which characters are of the most phylogenetic significance in the scarabaeid larvae.

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GROWTH AND FEEDING BEHAVIOR OF LARVAE OF CTENICERA AERIPENNIS DESTRUCTOR (BROWN) (COLEOPTERA: ELATERIDAE).

I. EFFECTS OF CARROT SLICES AND SEEDS OF WHEAT, FLAX, BARLEY, RYE, AND ALFALFA[1]

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Strickland (1933, 1939) studied the effects of various natural foods on the survival of larvae of *Ctenicera aeripennis destructor* (Brown), and Evans and Gough (1942) investigated some of the factors that influence growth of larvae of *Agriotes* spp. Kosmachevskii (1950) reported a diapause condition when larvae of *Agriotes meticulosus* Cand. were reared in washed sand in test tubes with germinating wheat seeds for food. This is a report on the growth and the feeding behavior of larvae of *C. a. destructor* reared on carrot slices and seeds of wheat, flax, barley, rye, and alfalfa provided individually and in combination.

MATERIALS AND METHODS

Larvae of *C. a. destructor* were collected from fields in the Saskatoon area during the summer and early autumn. They were stored at 35° F. for 5 to 7 months in waxed cardboard cartons containing moist soil. The larvae were divided arbitrarily into two groups according to weight: medium-sized, under 20 mg.; and large, over 20 mg. The average initial weight for each group was 11.2 and 40.4 mg., respectively, when individual foods were provided, and 7.3 and 36.8 mg. when six foods were present.

One tablespoonful of oven-dried clay loam soil brought to 27 percent moisture saturation with distilled water was added to individual screw-cap vials, 1 inch in diameter and $2\frac{3}{4}$ inches high. One seed of wheat, barley, or rye, five seeds of flax or alfalfa, or half a slice of carrot $\frac{1}{4}$ inch thick was added to each vial when one food was supplied to the larvae. However, only one seed of each was added with the carrot slice when a combination of foods was given to them. One larva was added to each vial after having been weighed to the nearest 0.1 mg. Each vial was closed with a screw cap and was placed in a constant temperature cabinet at 68±2° F. and 50±5 percent relative humidity. The tests carried out with each food and with the combination of foods for larvae of both sizes were replicated four times, each replicate consisting of 10 larvae initially.

Each week for 30 weeks, the larvae were checked for mortality and were weighed. The soil was examined for exuviae, each food source was inspected for evidence of feeding, and freshly prepared soil and new food were added to the vials.

An arcsine transformation was applied to the data. Differences between effects of various foods on molting, feeding, survival, and change in weight were determined by analysis of variance at the 5 percent level.

TABLE I

AVERAGE WEEKLY PERCENTAGES AND RANGES (ANGULAR TRANSFORMATIONS) OF SURVIVING MEDIUM-SIZED AND LARGE LARVAE OF *C. a. destructor* FEEDING ON VARIOUS NATURAL FOODS

Food	Medium-sized Larvae		Large Larvae	
	Average	Range	Average	Range
Rye	45 8	27 3–61 3	41 7	30 0–57 7
Carrot	45 5	28 7–64 1	37 2	0 0–77 1
Wheat	41 5	15 4–63 4	40 7	27 9–53 7
Flax	34 4	18 9–52 5	33 6	0 0–58 4
Barley	34 3	17 6–51 3	26 4	0 0–34 8
Alfalfa	32 1	14 4–56 3	30 2	18 1–48 7
Combination	30 9	0 0–62 3	27 1	0 0–73 5
L.S.D. at 5% level	4 0		10 6	

RESULTS

Each week, about 45 percent of the larvae fed when reared on rye, carrot, or wheat and about 30 percent when reared on flax, barley, or alfalfa, or on a combination these six foods (transformed values, Table I). When the larvae were provided with a choice of food, they attacked a single food in 51 percent of the cases. Medium-sized larvae

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TABLE II

AVERAGE WEEKLY PERCENTAGES (ANGULAR TRANSFORMATIONS) OF MEDIUM-SIZED AND LARGE LARVAE OF *C. a. destructor* FEEDING ON SPECIFIC FOODS WHEN PROVIDED WITH A CHOICE OF SIX FOODS

FOOD	MEDIUM-SIZED LARVAE		LARGE LARVAE	
	One Food	More Than One	One Food	More Than One
Carrot	27 0	35 3	40 6	30 4
Rye	24 8	36 5	15 2	38 9
Wheat	46 7	42 3	13 2	50 4
Barley	6 0	20 9	7 9	16 7
Flax	5 6	14 9	10 2	13 8
Alfalfa	1 6	0 6	0 0	1 2
L.S.D. at 5% level	9 6	14 6	11 2	15 3

fed more frequently on carrot, rye, and wheat and large larvae oftener on carrot than on the others when a single food was eaten (Table II). However, none of the larvae fed on a particular food to the exclusion of the others throughout the experiment. Instead, they effected a mixed diet for themselves. Both sizes fed most frequently on wheat-rye, wheat-carrot, and rye-carrot combinations when two foods were eaten and on the wheat-rye-carrot combination when three foods were attacked. Medium-sized larvae included flax with these when they fed on four foods, but large larvae included either flax or barley. Both were eaten when the larvae fed on five foods.

The total number of molts per larva varied from none to 8 and averaged 3 when medium-sized larvae were reared on single foods, and from 1 to 4 and averaged 2 on a combination of foods. In both cases, the intermolt period ranged from less than 1 week to more than 30 and averaged about 5. Analysis of variance showed that neither diet nor incidence of feeding affected the average weekly incidence of molting of medium-sized larvae (Table III). When large larvae were reared on single foods, the total number of molts per larva varied from none to 6 and averaged 2, and on a combination of foods, from none to 3 and averaged 1. In the first instance, the intermolt period lasted from less than 1 week to 26 weeks; in the second, from less than 1 to more than 30 weeks. The intermolt period for larvae reared on a combination of foods was 11 weeks, 3 weeks longer than for larvae reared on a single food. Significantly more molting occurred when large larvae fed on alfalfa than when they fed on any other food except rye (Table III). The number of larvae feeding per week was unaffected by the number molting, except in the case of large larvae feeding on alfalfa (Table III), for which the regression coefficient was negative.

In about 30 percent of the cases, medium-sized larvae reared either on a single food or on a combination of foods fed during the week previous to that in which a molt occurred. Fifty-nine percent of those reared on a single food and 79 percent on a combination fed during the week after that in which a molt occurred. Molting and feeding were recorded during the same week in 11 percent of the cases of larvae feeding on one food and in 20 percent of the cases on a combination. Large larvae fed during the week before that in which a molt occurred in 24 percent of the cases on a single food and 6 percent on a combination of foods. In 48 and 76 percent of the cases, repectively, the larvae fed during the week after that in which molting occurred. Molting and feeding during the same week was recorded in 10 percent of the cases, both on a single food and on a combination.

Medium-sized larvae provided with alfalfa seed gained least (Table IV) and those with carrot and with the combination of six foods gained most. Large larvae reared on rye, barley, wheat, or alfalfa gained significantly less weight than those reared on carrot slices or on the combination.

TABLE III

AVERAGE WEEKLY PERCENTAGES (ANGULAR TRANSFORMATIONS) OF SURVIVING MEDIUM-SIZED AND LARGE LARVAE OF *C. a. destructor* MOLTING AND COEFFICIENTS FOR REGRESSION OF INCIDENCE OF FEEDING ON INCIDENCE OF MOLTING

FOOD	MEDIUM-SIZED LARVAE		LARGE LARVAE	
	Average	Coefficient	Average	Coefficient
Wheat	19 5	−0 5174	13 8	−0 8565
Flax	19 5	0 1831	13 3	−0 7581
Barley	17 7	0 1588	14 4	−0 4799
Rye	18 7	−0 5565	15 9	−0 1807
Alfalfa	20 2	−0 3692	18 3	−1 009*
Carrot	18 2	−0 3705	12 0	−0 0445
Combination	15 7	0 7895	11 3	0 0160
L.S.D. at 5% level	——		3 7	

*Significant at 5% level.

Those reared on flax gained significantly less than those on the combination, but not significantly less than those on carrot.

Individuals varied greatly in weight gained. The medium-sized larvae fed on alfalfa seed did not increase in weight over the whole period. Others on the same food gained 1.1 to as much as 14.4 times their initial weights. One larva reared on carrot slices attained a final weight of 84.2 times the initial, in comparison with 1.9 times for another on the same food. Large larvae behaved similarly, except that the range of variation was much narrower.

The gain in weight was independent of the number of molts for larvae of both sizes. One medium-sized larva did not molt during the investigation, but increased in weight 5.6 times. The two on alfalfa seed that did not change weight molted 3 and 4 times, respectively, or as often as the individual that gained the most weight. Another larva molted 8 times, but at the end of the investigation weighed only 8.3 times its initial weight.

Medium-sized larvae survived equally well on all foods (Table IV). However, large larvae survived best on alfalfa or on the combination.

TABLE IV

AVERAGE CHANGE IN WEIGHT IN MILLIGRAMS PER MILLIGRAM OF INITIAL WEIGHT AND ANGULAR TRANSFORMATIONS FOR PERCENTAGES OF MEDIUM-SIZED AND LARGE LARVAE OF *C. a. destructor* SURVIVING ON VARIOUS NATURAL FOODS

Food	Medium-sized Larvae		Large Larvae	
	Change in Weight	Survival	Change in Weight	Survival
Carrot	10 00	34 6	0 94	47 9
Flax	6 44	41 5	0 56	43 3
Barley	4 69	47 9	0 11	47 9
Wheat	3 62	34 7	0 28	53 4
Rye	2 96	32 3	−0 03	46 4
Alfalfa	1 75	39 1	0 19	62 1
Combination	10 36	60 6	1 20	69 7
L S D. at 5% level	7 91		0 51	16 1

DISCUSSION

Larvae of both sizes fed regularly and frequently on all foods throughout the test. In no case was there a period of intense feeding followed by a prolonged period of complete rejection of food. This suggests that the relative immunity from wireworm attack of a reseeded crop is influenced by factors other than the nutritional state of wireworm larvae.

Because observations were made at weekly intervals, the feeding records indicated only that feeding had occurred during the week. A larva may have fed once or several times during that period. The latter did occur in about half the cases recorded when the six foods were provided at the same time, but did not occur when flax or alfalfa were the only seeds provided.

The size of the food apparently did not influence the feeding of the larvae. Carrot slices were generally larger than kernels of wheat or barley, and they in turn were larger than kernels of rye, flax, or alfalfa. Medium-sized larvae fed to the same extent on carrot, wheat, and rye when they were supplied singly or in combination with the other foods; large larvae fed more on carrot than on any of the other foods when the six were provided at the same time. Had the increased feeding on carrot been caused by the size of the carrot slice, it should have occurred also when medium-sized larvae were studied.

Failure to feed on alfalfa may have been because of the hardness of the seed coat, although this seems unlikely. If hardness of the seed coat were an important factor, far more feeding would have been recorded with carrot slices than with rye seeds. This was not the case, especially with medium-sized larvae.

In this investigation, the larvae usually attacked the germ end of the seed to gain entry to it. This happened either because the embryo end was soft in comparison with the rest of the seed or because chemical substances produced by the growing embryo were attractive to the larva. However, entry was not always gained through the germ end. On all seeds, but especially on flax, entry was often gained by larvae drilling through the middle of the seed. Whether the endosperm was eaten was apparently dependent on the length of time that the wireworm larvae fed. Some seeds had only the embryo destroyed; others were completely hollowed out, so that only the seed coat remained.

The foods used in this investigation were chosen in an attempt to provide the larvae with foods of different nutritional values. Those of high nutritional value should have produced better growth than those of low nutritional value. There was no evidence that diet influenced the incidence of molting of medium-sized larvae, although it affected growth as measured by gain in weight. Alfalfa and rye caused large larvae to molt more frequently than the other foods, but did not increase their rate of growth. The increase in the number of large larvae molting on alfalfa was accompanied by a decrease in the number feeding. Apparently this decrease was not caused by the food, but rather by the fact that the larvae molted so frequently.

Evans and Gough (1942) noted that larvae of *Agriotes* spp. gained weight during periods of fasting. Observations during this investigation indicated that larvae of *C. a. destructor* may feed and lose weight, gain weight without feeding, or molt and gain weight without feeding. Ecdysis did not always indicate a change to a more

mature form of larva. Because of the great variation in behavior, especially of medium-sized larvae, extreme caution should be employed before estimating the maturity of the larva either by size or by number of molts.

Strickland (1939) stated that only monocotyledonous vegetation fulfilled the food requirements of young larvae of *C. a. destructor*. Evans and Gough (1942) observed that wheat was the most suitable food for rapid growth of larvae of *Agriotes* spp., carrot less suitable, and bean least suitable. In the present investigation, dicotyledons were as effective as monocotyledons in the diet of larvae of *C. a. destructor*. Larvae of *C. a. destructor*, unlike those of *A. meticulosus* (Kosmachevskiĭ, 1950), did not enter diapause when they fed on germinating wheat seeds.

SUMMARY

Field-collected medium-sized (under 20 mg.) and large (over 20 mg.) larvae of *C. a. destructor* were reared for 30 weeks on carrot slices and on seeds of wheat, flax, barley, rye, and alfalfa provided individually and in combination. A greater percentage fed weekly on rye, carrot, or wheat than on flax, barley, alfalfa, or a combination of foods. Given a choice of six foods, the larvae fed more frequently on carrot, rye, or wheat, or on some combination of the three than on any other food or combination of foods. The larvae attacked one food as frequently as several, but did not feed on a particular food to the exclusion of others; by this means, they effected a mixed diet. This diet influenced the number of larvae that fed during the week before, the week after, and the week of molting.

Individuals varied greatly in weight gained, and the gain was independent of the number of molts and of the incidence of feeding. They fed and lost weight, gained weight without feeding, and molted and gained weight without feeding. Ecdysis did not always indicate a change to a more mature form of larva.

Carrot and wheat alone or in combination with the other foods produced large increases in weight. Rye was a poor food for the larvae when supplied alone, but did not affect them adversely in combination with carrot and wheat.

Survival of medium-sized larvae was unaffected by the mixed diet. However, large larvae survived better on it than on wheat, flax, barley, rye, or carrot.

Although other workers found that monocotyledons were better than dicotyledons as food for this species and for *Agriotes* spp., these results show that dicotyledons satisfied the food requirements of larvae of *C. a. destructor* as well as monocotyledons. No evidence was obtained to suggest that feeding on germinating wheat seeds induced diapause in larvae of this species.

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APHIDS ATTRACTED TO LIGHT TRAPS[1]

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Very little information appears in literature concerning the response of aphids to light. It is generally believed that they are day-flying insects and do not respond to light. Broadbent (1947) was the first to point out that they fly throughout the night and are freely attracted to light. The writers have determined that some, but not all species of aphids are attracted to light. Broadbent took 3,400 aphids from a single trap during a period of four years, 1933 to 1936. The catches began in May, gradually increased, reached a peak in July and August and then declined. He found that they were active at temperatures above 47° F. and that rain during the afternoon, the night or both, reduced the catches considerably. The writers believe that moderate rain during the night, providing the temperature is above 50° F. increases the catches of aphids. According to Broadbent, wind during the day or evening affected the catches more than wind during the night. Although he captured a large number of aphids it is unfortunate that he did not indicate the species involved. Milne (1945) records *Aphis maidis* from a light trap, the only specific record to date.

The aphids recorded in this article were taken from one or two light traps operated by the senior author in a small area in State College, Pa., during the summer of 1953, 1954, 1955 and 1956. These traps were hung about six feet above

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the ground[2]. Various types of traps and lamps were used; however, white tungsten-filament and ultraviolet lamps[3] were used most of the time. From these traps the junior author has identified 4390 individuals representing 55 species. Not all of the specimens collected were preserved and identified. When the collections were small, all were identified, but when the collections were very large, only representative samples were saved for identification. Table I, therefore is not intended to indicate the quantitative occurrence of individual species, although there are sufficient data to indicate trends. The traps were not operated during the last week of July and the first two weeks of August, hence data are missing for these periods.

From our records it is apparent that all species of aphids do not respond equally to lights. *Rhopalosiphum fitchii* (Sands), *Macrosiphum granarium* (Kirby) and *Eriosoma americanum* (Riley), all common species, were taken in large numbers. On the other hand, *Aphis pomi* DeG., *A. spiraecola* Patch, and *Macrosiphum solanifolii* (Ashm.) also common species, were seldom taken at the lights. It should also be noted that the hosts of these three species were present in the immediate area where the traps were operated.

The response of *Macrosiphum granarium* (Kirby) to light was phenomenal. This species has never been observed frequently in Pennsylvania. It was taken in light traps more frequently than any of the other species. Strangely,

TABLE I

NUMBERS OF APHIDS IN LIGHT TRAPS, STATE COLLEGE, PA. 1953, 1954, 1955, 1956

MONTHS	MAY				JUNE				JULY			AUGUST		SEPTEMBER				OCTOBER				NOV.	
WEEKS	1	2	3	4	1	2	3	4	1	2	3	3	4	1	2	3	4	1	2	3	4	1	TOTALS
Cinara piliconis (Hartz.)					1	1	1		3														6
Calaphis betulaecolens (Fb.)							1	2					1	1									5
Euceraphis betulae (Linn.)														1	1				3	1			6
Euceraphis mucidus Fb.					2	2	2									1							7
Myzocallis alhambra Davds.					10		2																12
Tuberculatus punctatella (Fb.)					10	14	10	6	1														41
Tuberculatus ulmifolii (Monl.)				5	22	4	6	13	1														51
Drepanaphis acerifoliae (Thms.)						2	1	1				1		1									6
Drepanaphis sabrinae Miller													1	4									5
Drepanosiphum platanoides Schrk.				2				8	5	2		1	5	10				3					36
Anuraphis bakeri Cowen							1	0	2					2				4					9
Anuraphis rosea (Baker)				5		1	7	4	3		3				1								24
Cavariella aegopodii (Scopl.)					2													8					10
Rhopalosiphum fitchii (Sand.)			2	17	29	193	46	268	42		2		10	12	1	2	11	106	2	25	65	169	1002
Macrosiphum granarium (Kby.)	27	6	5	339	58	93	114	208	4		10			13	42	3	31	189	0	3	25	272	1442
Macrosiphum pisi (Harris)		3	0	118	6	23	56	13	1				1	27	4	8	7	5					272
Macrosiphum rosae (L.)						1	2	5	10														18
Myzus persicae (Sulz.)				2		2	22	4	1					1	3	2	8	16	0	4	9	6	80
Capitiophorus braggii (Gill.)					1									3	9	1	7	42	3	11	63	108	248
Capitophorus hippophaes (Walk.)																		18	3	1	9	6	37
Eriosoma americanum (Riley)	6	22	16	42	48	216	315	75	25		3			15	7	6	5	19	0	18	22	2	862
Eriosoma lanigerum (Hausm.)													5										5
Prociphilus imbricator (Fb.)													1	4	3	2				1			11
Thecabius populi-monilis (Riley)		1				7								4		7		3		13	46	64	145
Totals 24 species	33	32	23	530	189	559	586	607	98	2	18	2	24	97	71	33	69	413	8	79	240	627	4340

Table I indicates two rather distinct periods (1) when aphids were flying, during the Spring and early summer, chiefly May and June and (2) during the Fall, chiefly September and October. The figures presented by Broadbent do not show such periods; however his records are for Derbyshire, England.

[2]Davis (1954) stated that the highest catch of aphids, 66 percent, was taken in pans at the ground level and that 15, 9, 6 and 4 percent were taken at heights of 5, 10, 15 and 20 feet, respectively. This might indicate that light traps would catch more aphids if operated at a lower level.

[3]15-watt G.E. black-light fluorescent lamps whose radiation peaks around 3500 Angstroms.

no wheat or grain occurs within the area where the traps were operated.

The large collections of *Rhopalosiphum fitchii* (Sands) and *Eriosoma americanum* (Riley) would be expected since apple and elm occured adjacent to the trapping area.

Some relatively rare species were taken in light traps. Two specimens of *Prociphilus venafuscus* (Patch) were taken in October and November. It has never been taken in Pennsylvania before. *Rhopalosiphonus latysiphon* Davds., another rare species was taken in June. The most remarkable catch was *Thecabius populimonilis* (Riley) of which 145 specimens were taken throughout the summer from May to November.

This was a surprise because the species is known to occur only on poplar which does not occur in the immediate area where the traps were operated.

As previously indicated, aphids were collected by various types of traps and lights. In most cases white and ultraviolet lamps were used. A yellow lamp was used in one case only. Table II indicates the preference of aphids for ultraviolet.

TABLE II

NUMBERS OF APHIDS ATTRACTED TO DIFFERENT LIGHTS STATE COLLEGE, PA.

PERIOD	100 WATT YELLOW	100 WATT WHITE	100 WATT UV BH[4]	15 WATT UV BL. LIGHT FLUORESCENT
6/15-7/1	6	105		
7/7-8/20		85	202	
7/15-18				201
8/10-21		26		
9/12-27		35		300

Identical traps were used for comparing yellow, white and ultraviolet lights of equal intensities. The 15 watt ultraviolet lamp required a slightly different type of trap. It is interesting to note that Broadbent (1948) and others have found that aphids are attracted more strongly to yellow than to white painted traps operated during the day time. The figures in the preceeding table indicate that aphids respond more freely to ultraviolet and white than to yellow light.

The figures for September 12 to 27 were even more convincing than appears in the table. A white light was used in the New Jersey light trap with an electric fan. A black-light fluorescent lamp was used in the standard Pennsylvania insect light trap with four aluminum baffles and a central tubular lamp. The two traps were operated adjacent to each other with the lamps scarcely more than two feet apart. Had the aphids been strongly attracted by the white light they would have been drawn in by the suction of the fan; however they definitely selected the Pennsylvania trap with the ultraviolet lamp.

Conclusions. Aphids generally respond to light. During four successive seasons 4390 individuals were taken representing 55 species. Not all species are strongly attracted. Some common species apparently are not attracted to lights. The majority respond more freely to utraviolet than to white or yellow light of approximately the same intensity. Light traps equipped with ultraviolet lamps are an excellent method of gathering records of certain species. They also yield records on rare species not taken by other collecting methods.

TABLE III

APHIDS OCCASIONALLY TAKEN AT LIGHT STATE COLLEGE, PA.

	NUMBER	DATES
Anoecia corni (Davds.)	3	Sept. Oct.
Longistigma caryae (Harris)	1	June
Lachnus salignus (Gmln.)	2	June Aug.
Myzocallis discolor (Monl.)	1	June
Myzocallis tiliae (L.)	2	Aug. Sept.
Melanocallis caryaefoliae Davis	1	June
Drepanaphis carolinensis Smith	1	Oct.
Drepanaphis monelli (Davis)	2	May
Drepanaphis parvus Smith	1	Sept.
Chaitophorus vimanalis Monl.	1	June
Periphyllus negundinus (Thms.)	2	Sept.
Clavigerus populifoliae (Fb.)	1	June
Clavigerus smithiae (Monl.)	1	Oct.
Aphis gossypii Glov.	1	June
Aphis helichrysi Kaltb.	2	May
Aphis mandis Fb.	2	Sept.
Aphis padi L.	1	June
Aphis pomi DeG.	1	May
Aphis spiraecola Patch	2	July
Hyalopterus pruni Geoffr.	4	June
Rhopalosiphonus latysiphon Davds.	1	June
Amphorophora rubicola Oestl.	1	June
Amphorophora vaccinii Mason	2	June
Macrosiphum ambrosiae (Thms.)	1	June
Macrosiphum pterocolens Patch	1	June
Macrosiphum solanifolii (Ashm.)	1	June
Myzus lythri (Schk.)	2	June
Capitophorus flaveolus (Walk.)	3	Aug. Sept.
Procaphilus venafuscus (Patch)	2	Oct. Nov.
Mindarus abietinus Koch	2	May June
Pergandeida trirhodus Walk.	2	May

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THE TAXONOMY OF THE FIELD CRICKETS OF THE EASTERN UNITED STATES (ORTHOPTERA: GRYLLIDAE: ACHETA)[1]

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INTRODUCTION

Orthopteran taxonomists are plagued with a number of genera containing species groups that defy subdivision by the time-honored criterion of morphological distinction. Although relatively few species of singing Tettigoniidae and Gryllidae have been described in the last fifty years from the eastern United States, an estimated 10 percent of the species in these groups are largely unrecognized because preserved specimens are morphologically indistinguishable. Many of these have been mentioned in the literature, and some have been recognized at one time or another as distinct species, only to be cast into synonymy or compromised as trinomials by subsequent authors. Many of them have been variously referred to as "physiological races," "ecological races," "biological races," "song forms," or by other more or less meaningless terms.

Our present knowledge of the relationships of many of these species is largely the result of the contributions of B. B. Fulton whose investigations have been characterized by field recognition of species on the basis of distributional, ecological, seasonal, and song relationships, followed by attempts to find morphological characters by which preserved specimens can be separated. Much of Fulton's work has been conducted with groups upon which supposedly exhaustive studies had already been carried out through morphological analysis of large series of preserved specimens.

However, even Fulton has hesitated to honor with formal nomenclature species which cannot be distinguished "on the pin." This stand has been supported by Hubbell (1954, 1956) and other orthopterists (personal communication), apparently on the grounds that (1) this is a relatively rare phenomenon, (2) only a small group of workers will be concerned with such situations, (3) a great deal of confusion would result if museum workers could not apply the proper specific names to specimens in their collections, and (4) in such cases the designation of the entire group by a single specific name and the use of colloquial names to distinguish the various species will suffice for most applied problems and museum collections.

The evidence indicates that the presence of morphologically indistinguishable species is not at all a rare phenomenon in the Orthoptera, and the work done by Fulton might well serve as an example for investigators puzzling over morphologically ill-defined forms in the non-singing Orthoptera with distributional, ecological, and seasonal relationships analogous to those ocurring between closely related species in the singing Orthoptera.

It is difficult to estimate the size of the group of workers which will be interested in a particular group of closely related species. There is a growing amount of published material dealing with the morphology, physiology, nutrition, and genetics of certain groups of Orthoptera in which the organisms involved were not and now perhaps cannot be identified because the investigator was not aware of the existence of several species in the group he was studying. Yet many morphologically ill-defined species are easy to separate when alive, in some cases even easier for the non-specialist than morphologically well-defined species, since their ecological, seasonal, and song relationships have been more thoroughly studied and are easier to interpret. If these closely related pairs and groups of species remain unknown to all but a handful of orthopterists, their potential usefulness to students of systematics and evolution will not be realized.

As with most biological phenomena, the problem of morphological distinctness is not always clear-cut. For example, if only one sex of a species can be identified, this still leaves specimens which cannot be determined. In the present study of the five species of *Acheta* in the eastern United States, certain pairs of species can always be distinguished "on the pin," at least in one sex. In others only certain percentages of the individuals can be distinguished. This paper is the first of a series concerning several such species groups whose relationships have been clarified through a study of sound production and associated behavior in the Orthoptera.

In addition to the type material of *A. fultoni*, representative male and female specimens and tape recordings of the calling songs of each of the species discussed in this paper will be deposited in the Museum of Zoology, Ann Arbor, Michigan. Representative male and female specimens will also be deposited in the United States National Museum and the Entomological Museum of The Ohio State University. An additional copy of the tape of the calling songs will be deposited with the Cornell Laboratory of Ornithology (Repository for the International Committee on Biological Acoustics). All the original recordings used for analysis are in the Library of Animal Sounds, Department of Zoology and Entomology, The Ohio State University.

[1]Accepted for publication March 19, 1957.

PRESENT STATUS OF AMERICAN *Acheta*

Native field crickets in the genus *Acheta* occur over most of North, Central, and South America, in the West Indies, the Galapagos, and on many other islands. Within this area they can be found almost everywhere except at high altitudes, in the far north, and in extremely moist situations. They are large, commonplace, easily captured insects with many prominent morphological features. Yet no satisfactory systematic analysis of even a small part of the genus has been produced. The first description of an American field cricket was published in 1775, and an almost uninterrupted flow of taxonomic papers dealing with the group has continued since. Forty-seven names had been applied to the American field crickets by 1903. Lutz in 1908, and Rehn and Hebard in 1915, after studying large series of specimens, concluded that only one highly variable species was represented. Since 1915 all the native field crickets have been lumped together under the name *Acheta assimilis* Fabricius,[2] the type of which is from Jamaica.

Practically all the early workers on this group considered morphology alone; such characters as size, coloration, wing venation, body proportions, number and relative lengths of tibial spines, and the length of the tegmina, wings, ovipositor, and hind femora were most often discussed. A wide range of variation exists in most of these characters. American field crickets range from 12.8 mm. to 28.8 mm. in body length, and from solid black to pale straw in color. In general, forms that are morphologically similar in different localities can be found in three types of habitats: (1) large, light-colored forms in sandy areas (beaches, sand dunes, and deserts), (2) small, black forms in deciduous forests, and (3) forms intermediate between these two extremes in grassy and weedy areas such as prairies, fields, pastures, roadsides, and lawns. Rehn and Hebard (1915) recognized this ecological distribution of morphologically similar forms, but because no discontinuities existed with respect to the characters they observed, described them (p. 294) as ". . . mere variations, the adaptations of this exceedingly plastic species to local environmental conditions." They further stated (p. 299) ". . . none of these are distinct either specifically or as geographic races, and really show only the various phases resultant from varied environmental conditions."

Blatchley (1903) was the only investigator to supply detailed biological information along with description of new species. A number of workers, such as McNeill (1889) in Illinois, Allard (1929) in Georgia and New England, Severin (1935) in South Dakota, Ball (1942) in Arizona, and Cantrall (1943) in Michigan, noted variations in life history and song in the field crickets they observed in these different parts of the country. In 1952, B. B. Fulton published a study of the field crickets of North Carolina in which he described four populations that differed in ecology, life history, song, and distribution, but had no distinguishing morphological features. These four populations failed to interbreed in fifty attempts. In 1953, when the present study was begun, Dr. Edward S. Thomas of the Ohio State Museum told this writer that he had known for some time that at least two different kinds of field crickets occurred in Ohio, with differences in ecology and song. The present study has revealed three species in northeastern United States, one of these in addition to the four discussed by Fulton (1952), making a total of five species known in eastern United States. In view of the complexity of the situation, it is not surprising that conventional taxonomic techniques have failed in its analysis. It is hoped that the results presented here will stimulate further study of this interesting genus in the great part of its range that is yet unexplored.

NOMENCLATURE AND SYNONYMY

There are forty-seven names available for American *Acheta* from descriptions based almost entirely on morphological characters (see Rehn and Hebard, 1915). For four of these descriptions the type locality given is simply "North America." Six descriptions were of specimens from western United States, and eight were of specimens from eastern United States. The rest were from Jamica, Bermuda, the Galapagos Islands, Guadalupe Island (Lower California), and various South American countries. Fifteen types have been examined by the writer, including all but three of the possible twelve from eastern United States. Information concerning the types of the remaining three (*G. luctuosus* Serville, *G. abbreviatus* Serville, and *G. scudderianus* Saussure) was obtained through correspondence with Dr. Lucien Chopard, Paris, and Dr. Charles Ferriere, Geneva.

In the course of this study several thousand specimens have been examined from all over the United States, and from Jamaica, Puerto Rico, Mexico, and several South American countries. The type of *A. assimilis* Fabricius (1775, Jamaica) has been lost, according to Dr. S. L. Tuxen, Copenhagen, where the Fabricius collection is on

[2]The sequence of changes in the genus name used for American field crickets (*Gryllus* Linnaeus, 1758, to *Gryllulus* Uvarov, 1935, to *Acheta* Fabricius, 1775) is discussed in detail by Gurney (1950, 1951). Since completion of this manuscript the writer has been informed by Dr. Gurney (in correspondence) that Chopard (Mem. Soc. Roy. Ent. Belgique 27: 153, 1955) has suggested that the present separation of American and some other field crickets and the European house cricket in the genus *Acheta* be dropped, and that the genus name *Gryllus* again be used for all the crickets in this general group. Dr. Gurney states, "I have decided to continue using *Acheta* until going into the matter further, but I expect the changes will eventually be accepted here because the genera [*Acheta* and *Gryllus*] have been rather artificial."

loan from the Zoological Museum at Kiel, Germany. This information has been confirmed by Dr. Tischler at Kiel. Specimens obtained from Jamaica through the kindness of Mr. Thomas Farr of the Science Institute at Kingston appear morphologically distinct from all the species discussed here, and Fabricius' description is so brief that it could be applied to almost any of the American species. From morphological comparisons alone it seems unlikely that any of our eastern species extend into Central or South America.

The types of the following species described from western United States have been seen by the writer: *G. personatus* Uhler, 1864, Kansas; *G. vocalis* Scudder, 1901, Palm Springs and Los Angeles, California; *G. integer* Scudder, 1901, West Berkeley to San Diego, California; *G. armatus* Scudder, 1902, Beaver Dam, Utah, Ehrenberg and Fort Whipple, Arizona; and *G. alogus* Rehn, 1902, Albuquerque, New Mexico. The type of the only other species described from this area, *G. lineaticeps* Stål, 1858, San Francisco, California, has been lost, according to Dr. R. Malaise of the Natural History Museum, Stockholm. *G. personatus* Uhler is quite distinct from all the eastern species, and seems to be recognizable in collections. Even Rehn and Hebard recognized the distinctiveness of this form, stating (1915, p. 294), that it "... constitutes the nearest approach to a geographic racial development." The taxonomic status of the other five species is not yet clear, but any that are the same as an eastern species here recognized are synonyms of a species described earlier; hence none of these western names need be considered as valid names of any of the eastern species. The synonymy which is given below is based on distribution, morphology, and life history, and discussions of the reasons for the less evident decisions will be found in the sections treating these characteristics.

Acheta pennsylvanica (Burmeister)

NORTHERN FIELD CRICKET

Gryllus pennsylvanicus Burmeister, 1838, p. 734, North America; Zoologisches Museum, Humboldt-Universitat zu Berlin (male lectotype here designated from syntypes; only labels are type label, catalogue number 983 (same as for other specimens in series), and determination labels as follows: *pennsylvanicus* Burm.*, *abbreviatus* Serv., *pensylv.* Timerm., *Gryllus* L. Burm.).

Gryllus luctuosus Serville, 1839, p. 335, North America; Museum National d'Histoire Naturelle, Paris (only a single female remains).

Gryllus abbreviatus Serville, 1839, p. 336, North America; Museum National d'Histoire Naturelle, Paris (only a single female with broken ovipositor remains).

Acheta nigra Harris, 1841, p. 123, no type locality given, but apparently New England; Museum of Comparative Zoology, Harvard College, Cambridge, Massachusetts (type probably destroyed; a few remnants of two specimens, a male and a female, remain, and an entry in Harris' notebook, "Autumn of 1834," indicates these specimens were from a fall population which would preclude their being any species other than *pennsylvanicus*).

Gryllus angustus Scudder, 1862, p. 427, Cambridge and Cape Cod, Massachusetts; Museum of Comparative Zoology, Harvard College, Cambridge, Massachusetts.

Gryllus neglectus Scudder, 1862, p. 428, Massachusetts and Cape Cod, Massachusetts; Museum of Comparative Zoology, Harvard College, Cambridge, Massachusetts.

Gryllus arenaceous Blatchley, 1903, p. 434, sand dune region of Lake County, Indiana; Entomological Collection, Purdue University, Lafayette, Indiana.

Acheta firma (Scudder)

BEACH CRICKET (Fulton, 1952)

Gryllus firmus Scudder, 1902, p. 294, Brookville, Indiana, Smithville and Pungo (not Dingo) Bluff, North Carolina; Georgia; Sanford and Key West, Florida; Museum of Comparative Zoology, Harvard College, Cambridge, Massachusetts. Lectotype here designated, male from Pungo Bluff, North Carolina. Scudder's Brookville, Indiana, specimen is *Acheta pennsylvanica* (Burmeister).

Acheta rubens (Scudder)

TRILLER FIELD CRICKET (Fulton, 1952)

Gryllus rubens Scudder, 1902, p. 294, Auburn, Alabama; Museum of Comparative Zoology, Harvard College, Cambridge, Massachusetts (description based on a single female).

Acheta vernalis (Blatchley)

NORTHERN WOOD CRICKET

Gryllus assimilis vernalis Blatchley, 1920, p. 704 (nom. nov.). Although Blatchley's holotype and allotype female, both from Crawford County, Indiana, are of this species, some of the specimens in his collection which he determined as *vernalis* are *A. fultoni* n. sp., described below.

Gryllus americanus Blatchley, 1903 (not Drury, 1773), p. 443, Crawford, Posey, Vigo, Putnam, Marion, Marshall, Wells, and Lake Counties, Indiana; Entomological Collection, Purdue University, Lafayette, Indiana.

Acheta fultoni n. sp.

SOUTHERN WOOD CRICKET

Holotype Male: Collected by the author in Hocking County, Ohio, Goodhope Township, Section 18, 4 June 1955, in a patch of leaf litter just outside an oak forest bordering an abandoned hillside field dominated by *Andropogon virginicus* L. Head, pronotum, and abdomen black; tegmina and cerci a rather uniform light brown; hind femora and all tibiae and tarsi a dull reddish. Pronotum narrowest near its anterior border; head narrower than pronotum; tegmina reaching to base of cerci; underwings completely hidden by tegmina. Otherwise not distinguishable from other American *Acheta* except by body measurements given below, and as shown in Figures 1–6, 16, and 17.

Allotype Female: Collected with holotype; same as holotype except tegmina lack reaching to base of cerci by about four abdominal segments; ovipositor reddish brown, darker near the base.

Measurements of Holotype and Allotype (in millimeters, made with ocular micrometer in binocular microscope): Body length, ♂, 15.8, ♀, 16.4; pronotal length, ♂, 3.5, ♀, 3.5; pronotal width, ♂, 5.2, ♀, 5.2; head width, ♂, 4.3, ♀,

4.3; length of hind femur, ♂, 10.3, ♀ 10.6; length of tegmina, ♂, 8.3, ♀, 9.0; length of ovipositor, 12.0.

Paratypes: Alexander Collection (collected by the author unless otherwise indicated: OHIO: type locality, 12 May 1954, 1♂ (E. L. Sleeper); 28 June 1954, 5♂; 4 June 1955, 26♂, 7♀; Fairfield Co., Madison Twp., Section 2, 5 June 1955, 2♂; 12 May 1955, 2♂; Adams Co., Jefferson Twp., Cedar School, 8 June 1955, 1♂. ILLINOIS: Jackson Co., about one mile southeast of Carbondale, 15 June 1955, 1♂; Giant City State Park, Jackson Co., 15 June 1955, 8♂, 3♀; Wolf Lake, Union Co., 15 June 1955, 3♂, 1♀. MARYLAND: Snow Hill, Worcester Co., 21 July 1956, 1♂; Whiton, Worcester Co., 21 July 1956, 1♂. NORTH CAROLINA (B. B. Fulton): Raleigh, 27 May 1929, 1♀; 3 June 1931, 1♂, 1♀.

This species is closest to *Acheta vernalis* (Blatchley) from which it differs in being slightly larger and in having brown tegmina and reddish appendages (at the least having reddish markings on the hind femora and usually on other appendages). As shown in figures 17 and 18, the tegmina in the male and the ovipositor in the female are both slightly longer in proportion to body length than in *vernalis*. The general body form, especially the distinctively narrow, retracted appearance of the head and the anterior narrowness of the pronotum is very similar to that of *vernalis*. Specimens from North Carolina, where this species occurs in pine-deciduous forests and along their borders, average larger and darker than those from Ohio and Illinois, where the species occurs in *Andropogon*-covered hillsides and along the borders of oak forests around such fields.

The life history, ecology, and general morphological characteristics of the southern wood cricket in North Carolina were described by Fulton (1952). This species is named in honor of Dr. Fulton, whose keen investigations first aroused the interest of the writer in the singing Orthoptera, and whose prompt and excellent replies to correspondence, and expert aid in locating and collecting singing Orthoptera have been invaluable in this and other studies.

The lack of understanding of American field crickets by early investigators is perhaps best revealed by the fact that an eastern species remains today to which none of the existing names, shuffled and reshuffled many times, seems to apply. This species was apparently first recognized by Fulton (1952) who called it the "wood

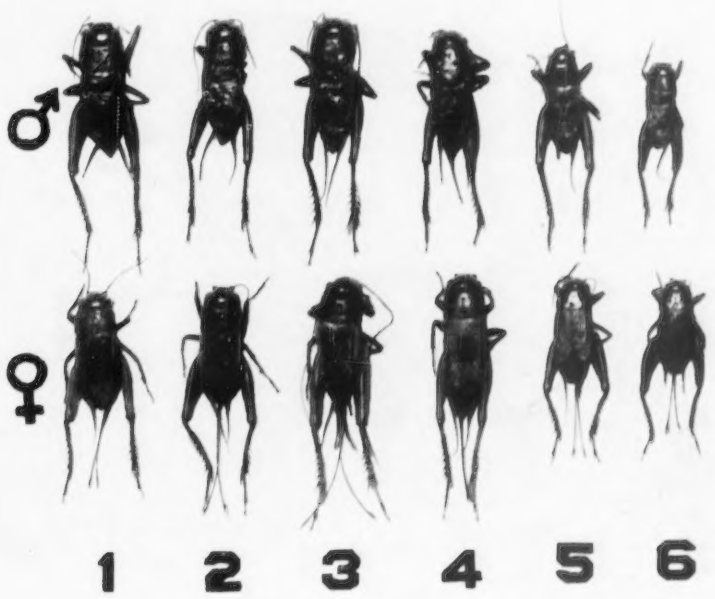

FIG. 1-6. Photographs of typical adult specimens of eastern field crickets. 1, *Acheta pennsylvanica* (Burmeister), spring brood; 2, *A. pennsylvanica* (Burmeister), fall brood; 3, *A. firma* (Scudder); 4, *A. rubens* (Scudder); 5, *A. fultoni* n. sp.; 6, *A. vernalis* (Blatchley); X 0.94.

cricket." The only earlier literature reference which seems to apply to this form is a remark by Rehn and Hebard (1915, p. 302) under the morphological class for which they include the names *luctuosus* Serville and *abbreviatus* Serville. They state, "The maximum of this [morphological] condition is found in material from the pine woods of southeastern United States." However, most of the specimens in the series determined by Rehn and Hebard as representative of this class are probably *rubens*. Furthermore, since field-collected specimens of this species never have the underwings exceeding the tegmina, and the single remaining female of *luctuosus* in Serville's collection is long-winged, this name could not apply. The measurements given for both *abbreviatus* and *luctuosus*, in addition to the almost solid black coloration, probably preclude their being any species but *pennsylvanica*. In correspondence, Dr. Lucien Chopard states, "I think that *abbreviatus* is the micropterous condition of *luctuosus*; both are very close to *pennsylvanicus*."

Only one name which could have been applied to a field cricket occurring in the eastern United States, *Gryllus scudderianus* Saussure, 1874, is omitted from the above synonymy. Saussure described eight species of field crickets and gave Mexico or various South American countries as type localities for all except this species, for which he gave only North America. Furthermore he named this species after Scudder, who did practically all his work on this group within the United States. In his discussion of the American species of *Gryllus*, Saussure placed *scudderianus* between *pennsylvanicus* Burmeister and *mexicanus* Saussure. In 1877 he placed *scudderianus*, along with *neglectus*, as a synonym of *abbreviatus* Serville. He described *scudderianus* from a male and a female, but according to Dr. Charles Fierriere, only a single female remains in the collection in the Natural History Museum, Geneva, Switzerland, with "Amer. Sept." and "Mr. H. deSaussure" the only information on the label. There is no indication as to how or where Saussure obtained this specimen. In general, the measurements Saussure gave for the type specimens fall somewhere near those obtained for specimens of *vernalis* and *fultoni* in the present study (body length, ♂, 14 mm., ♀, 15.0; ovipositor length, 15.0 mm.). Since both type specimens had brown tegmina and hind femora, it cannot be the same as *vernalis*. Practically all the available measurements differ from those given here for *fultoni*, and especially noticeable is the variation in proportionate length of body and ovipositor. It seems most probable that if Saussure's types came from eastern United States (and this is certainly doubtful), they represent dwarfed, atypical specimens of *pennsylvanica* (Burmeister), or *rubens* (Scudder). Very small specimens of these species have been seen, which nevertheless retain the general body proportions and other characteristics of the typical larger specimens. However, the deviation of *scudderianus* from the measurements given for species treated here, and the improbability of discovering further information about this name, in the opinion of the writer, justifies its exclusion from the present discussion.

DISTRIBUTIONAL RELATIONSHIPS

The known distribution of the eastern field crickets, based almost entirely on the collections and "listening" records of Fulton and the writer, is shown in figure 7. The Missouri, Arkansas, Louisiana, and Mississipppi records were obtained by Thomas J. Walker on a trip through this area, June 14–17, 1956. The Fentress and Cumberland County, Tennessee records for *vernalis* are from specimens in the University of Michigan Museum of Zoology collected by T. H. Hubbell on July 19, 1924, and July 13, 1922, respectively. The Fentress County, Tennessee, and Fulton County, Georgia records for *fultoni* are from specimens in the University of Michigan Museum of Zoology collected by T. H. Hubbell on June 10, 1925, and April 28, 1938, respectively. The Quantico, Virginia record for *fultoni* is from specimens in the Ohio State Museum collected by Edward S. Thomas on May 25, 1935. The general lack of records in the Kentucky-Tennessee-Alabama area is due to a lack of collecting in that area.

The most complete portions of the records are in the northward extension of *rubens*, the southward extension of *pennsylvanica*, the northward extension of *fultoni* in Ohio and on the Delaware peninsula, and the northward extension of *firma* along the Atlantic coast. The western limits are not known for any of the species. The northward and southward extensions of *vernalis* are not known. However, all of the 750 plus specimens from Michigan in the collection at Ann Arbor are *pennsylvanica*, and no specimens of *vernalis* have been found among hundreds of specimens seen from Wisconsin, Minnesota, Canada, and the New England states.

To avoid the possibility of error, distribution records based only on specimens seen in collections have not been included for *pennsylvanica*, *rubens*, or *firma*. The morphological variability and overlap of the specimens which would be referred to these three species suggests at least the possibility that additional species not encountered in this study may occur in eastern United States. Consequently, for all specimens determined as belonging to one of these three species, either the habitat or the song is known, in addition to the collection date and locality.

ECOLOGICAL RELATIONSHIPS

A. pennsylvanica occurs in all kinds of grassy situations, such as fields, pastures, weedy areas, roadsides, and lawns, and it is the most abundant and continuously distributed field cricket in

northeastern United States. In southeastern United States it is replaced in similar situations by ***rubens***. These are probably the only field crickets in eastern United States which occur in appropriate places and achieve sufficient abundance to become economic pests. They are also much easier to collect than any of the other species, and therefore dominate collections of

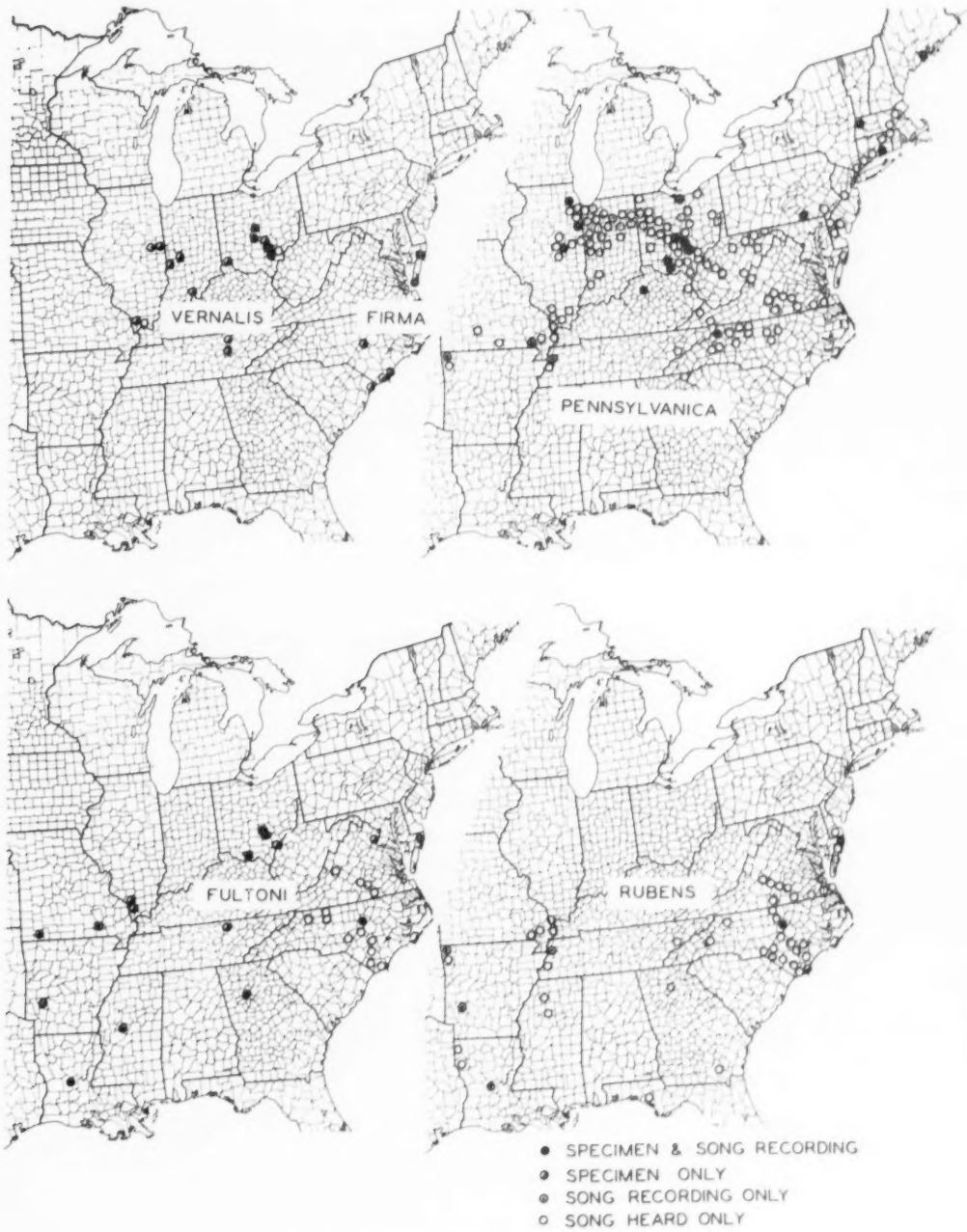

FIG. 7. Distribution of eastern field crickets.

eastern *Acheta*. This is perhaps one of the most important reasons for the confusion in early attempts to split this genus into species on the basis of morphological characters. Lutz (1908) placed great emphasis on the fact that the extremes of the characters he studied were represented in relatively few specimens, as one would expect in a single, highly variable population. He states (p. 6), "Typical specimens of *americanus* [*vernalis*] and *firmus* are rare for the same reason that very short men and very tall men are rare." The present study indicates that they are rare *in collections* because of less likelihood of encountering them and greater difficulty in capturing them. Of approximately 2700 eastern field crickets examined in six collections in eastern United States, there were about 1900 *pennsylvanica*, about 750 *rubens* and *firma* combined, 72 *vernalis*, and 24 *fultoni*. This does not include the collections of Fulton and the writer.

As stated by Fulton (1952, p. 285), *firma* inhabits "the flat sandy area bordering the ocean beaches. This may be grassy or shrubby or largely bare sand, but is not wooded." Later Fulton found chirping crickets in inland sand hills in Harnett and Hoke Counties, North Carolina, which produced fertile offspring in crosses with ocean beach crickets (personal communication). Audiospectrographic analysis has shown these island specimens to have the same song as the ocean beach crickets.

The distribution of *firma* along the Gulf coast and inland in that area is unknown. However, crickets morphologically resembling beach crickets have been seen in collections from inland locations in Mississippi, Alabama, and Georgia. Light-colored crickets occur in sandy areas around Lake Michigan and Lake Erie, along the Illinois and Mississippi Rivers, and probably in similar areas all over eastern United States. Such specimens were responsible for Blatchley's description of *arenaceous* from Lake County, Indiana. However, crossing experiments and song analysis have revealed light-colored specimens collected at Dunes State Park, Indiana, along Lake Michigan, and Cedar Point, Ohio, along Lake Erie, to be conspecific with the more commonly encountered, darker-colored specimens

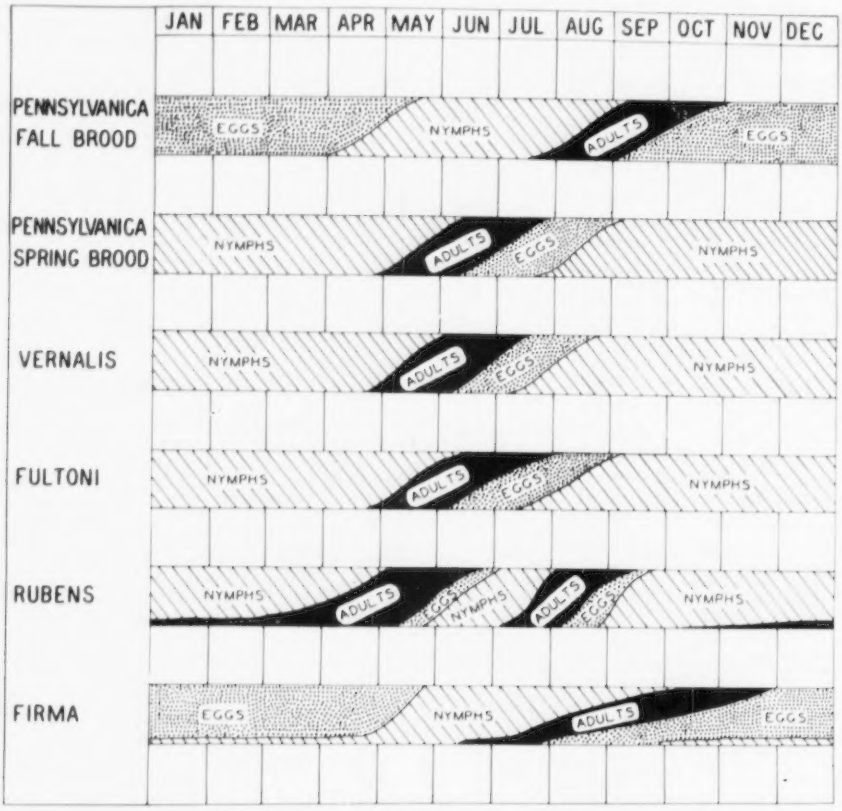

FIG. 8. Seasonal life histories of eastern field crickets.

of *pennsylvanica*. Such specimens apparently represent "adaptations . . . to local environmental conditions," such as are referred to by Rehn and Hebard (1915, p. 294). The morphological variants from inland localities in Mississippi, Alabama, and Georgia, however, remain to be checked, and may represent additional species to those discussed here.

A. vernalis is found in leaf litter in deciduous forests. In North Carolina and Virginia, where *vernalis* does not occur, this habitat is occupied by *fultoni*, which is abundant in deciduous and pine-deciduous forests, and along the forest borders. Both *vernalis* and *fultoni* occur in southern Ohio, Indiana, and Illinois. Here, however, *fultoni* is chiefly an inhabitant of dry, *Andropogon*-covered hillsides. In southeastern Ohio it is found almost exclusively in old fields, 10 to 30 years

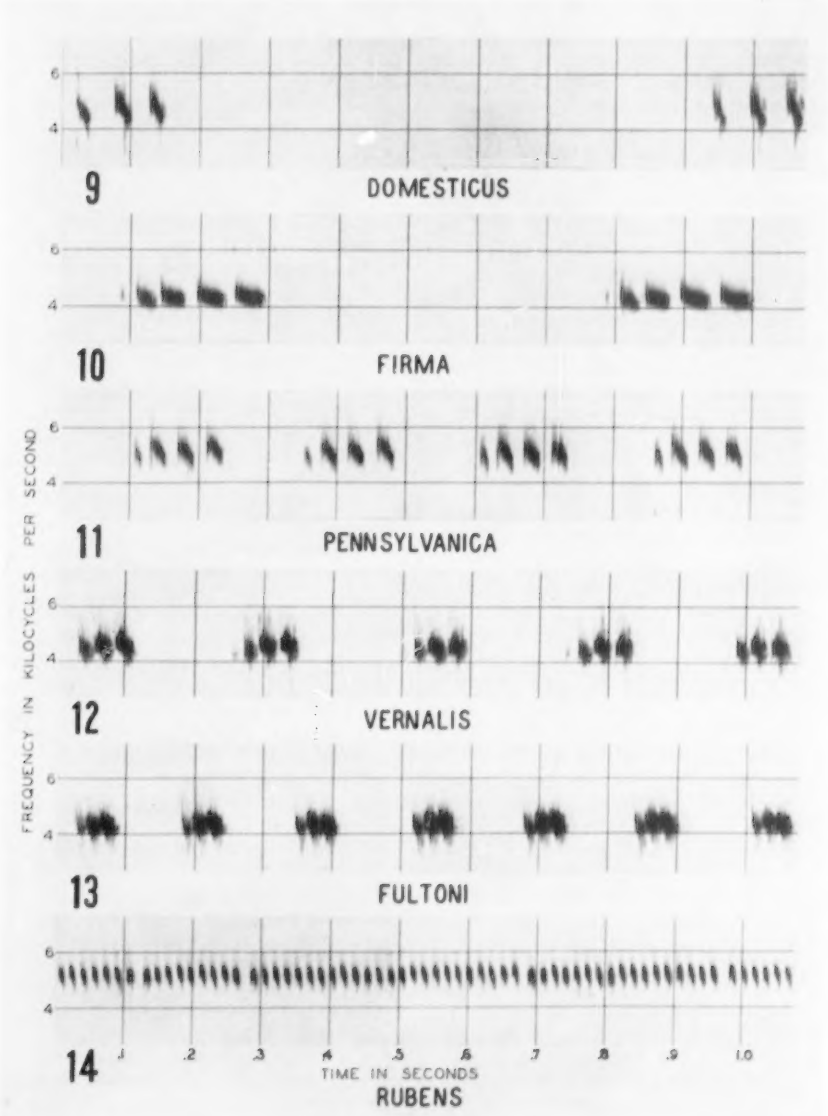

FIG. 9–14. Sample audiospectrographs of typical calling songs of *Acheta* species. 9, *Acheta domesticus* (Linnaeus); 10, *A. firma* (Scudder); 11, *A. pennsylvanica* (Burmeister), spring brood; 12, *A. vernalis* (Blatchley); 13, *A. fultoni* n. sp.; 14, *A. rubens* (Scudder).

abandoned, which are dominated by *Andropogon virginicus* L. In southwestern Ohio and west to along the Mississippi River bluffs in southern Illinois, *fultoni* occurs in the hilltop praire openings dominated by *Andropogon scoparius* Michx. In both Illinois and Ohio, *fultoni* is often more abundant along the forest borders around such fields than it is out in the fields themselves. In Maryland, Virginia, Arkansas, and Missouri, *fultoni* has been found both in *Andropogon* fields (and similar grassy areas) and in leaf litter in woods. The factors involved in this ecological variation remain to be elucidated. However, morphological comparisons, song analyses, and a single successful crossing experiment (between an Ohio female and a North Carolina male) seem to indicate that a single species is involved, with geographic variation in habitat and in certain song characteristics which will be described later.

A. fultoni and *pennsylvanica* overlap but little with *vernalis* where their habitats meet in Ohio. On the other hand, *fultoni* and *pennsylvanica* occur together in the same fields in southern Ohio, and appear to be randomly intermixed in such areas. *A. rubens* and *firma* have been found in mixed colonies at Kure Beach and Carolina Beach, North Carolina. In Worcester County, Maryland, *rubens*, *firma*, and *fultoni* were found together along the border of a wheat field, and *pennsylvanica* was collected only a few yards away along a grassy roadside.

SEASONAL LIFE HISTORIES

The seasonal relationships of the eastern field crickets are indicated in figure 8. The diagrams for *rubens* and *firma* are redrawn from Fulton (1952). *A. fultoni* has approximately the same life history in North Carolina as in Ohio.

No way of distinguishing the spring and fall broods of *pennsylvanica* in Ohio has been found other than the differences in their life histories, and a slight difference in the relative length of the body and the ovipositor in most females (fig. 18). One brood overwinters as partly grown nymphs, one or two instars from adulthood, and produces adults which sing from early May until about mid-July. The other overwinters in the egg stage and produces adults which sing from mid-July until frost. These two broods occupy the same habitat, a fall male often moving into the same burrow or niche inhabited by a spring male earlier in the season. There is a slight overlap of adults in mid-July. Although very few adults can be seen or heard at this time, there were at least a few singing males on the Ohio State University campus every night from May to October during the three years of this study. The end of the spring brood and the beginning of the fall brood are difficult to determine, but are indicated by the gradual disappearance of singing males in familiar locations, and the appearance of numbers of singers in new locations. Adult male field crickets are sedentary, ordinarily remaining in and singing from the same location throughout their adult lives.

There are indications that offspring of the spring brood of *pennsylvanica* never mature in fall in Ohio. No adults or last instar nymphs have ever been taken in early winter. Last instar nymphs of the fall brood can be found on the Ohio State University Campus until mid-September, but later in the fall only adults of the fall brood and young nymphs of the spring brood, about three instars from adulthood, can be found. In the laboratory, the offspring of Franklin County, Ohio, spring adults mated on May 4, started maturing as early as August 12. However, a few individuals in this group had not yet reached the last nymphal instar by mid-October. Another pair of spring adults from Piatt County, Illinois, was mated on June 6, and removed from the cage on July 11. Their offspring began maturing in October, but the last did not mature until the following April. Neither *fultoni* nor *vernalis* mature in the fall in Ohio, but both produced offspring from early May matings in the laboratory which began maturing in mid-August and early September, respectively. Several factors may be involved in these discrepencies between field and laboratory observations, such as temperature, diet, photoperiod, and water availability.

Fulton's findings in North Carolina indicated that offspring of spring adults of *pennsylvanica* may sometimes mature in the fall, while Cantrall (1943) failed to obtain any fall adults from seven pairs of spring brood adults caged outside on June 9, in Michigan. On the George Reserve, Michigan, only about five percent of the total number are spring crickets, a result, Cantrall believes, of high mortality among overwintering nymphs. In central Ohio, no noticeable difference in numbers occurs between the spring and fall broods. These two broods may interbreed in mid-summer, or possibly in fall in the southern part of their range, or it may be that they have been isolated such a short time that no noticeable differences have yet appeared between them. Certainly more investigation is needed to clarify their relationship.

A. vernalis and *fultoni* overwinter as nymphs one or two instars from adulthood, and during the three seasons that this study has been in progress, they have matured a week or two earlier than *pennsylvanica* in southern Ohio, beginning to sing in late April or early May. The adults disappear in late July and early August. In North Carolina, Fulton (1952) says *fultoni* sings from mid-April until early August. The writer collected an adult male and heard several singing on August 10, 1955, near Raleigh, North Carolina. One of the males collected was mated with a female from Hocking County, Ohio, on August 18, and was

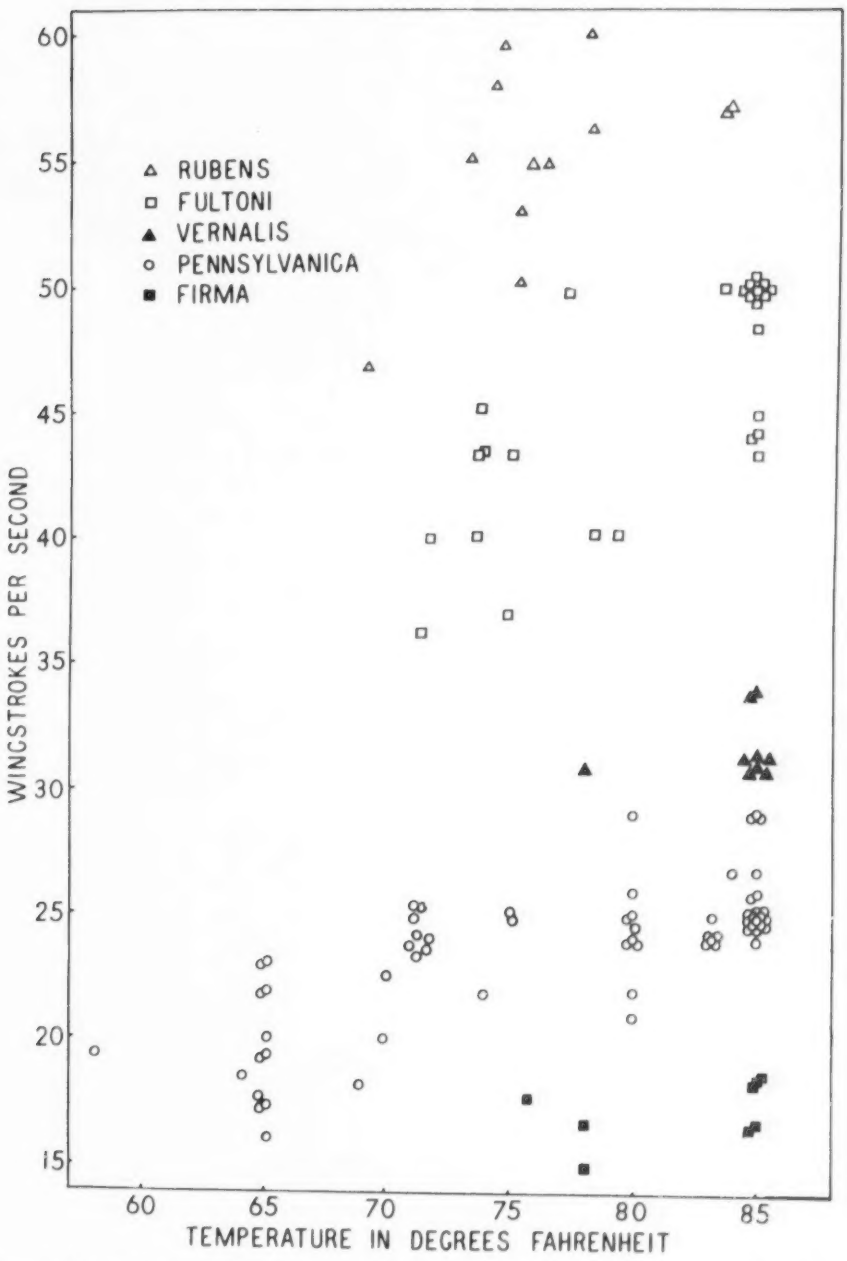

FIG. 15. The effect of temperature on rate of wing-stroke in the calling songs of eastern field crickets.

still alive on September 30. Offspring from this mating appeared on September 30.

SONG RELATIONSHIPS

Geographic variation in the songs of field crickets was first noted by Allard (1910). Fulton (1952) first used it as a taxonomic character in this group. In the singing Gryllidae, Tettigoniidae, and Cicadidae, song differences have been responsible for the initial recognition of many undescribed species (Davis, 1922; Fulton, 1930; Pringle, 1955; Thomas and Alexander, 1957). As remarked by Dr. Edward S. Thomas in conversation with the writer, to one who has studied these groups in the field it is almost a foregone conclusion that two insects which occur in the same area and have different songs are different species.

As with most singing Tettigoniidae and Gryllidae, male field crickets ordinarily space themselves in the field and remain in one spot all their adult lives. A large part of the time they produce a sound which is generally referred to as the calling song (also solitary or common song). This is the only sound which will be discussed here. The variety of sounds made by field crickets in other situations is discussed by Alexander (1957). The calling song attracts the adult females which move toward and locate the males. The roving females are seen much more often in the field than the sedentary males, and dominate collections for this reason.

Tape recordings and audiospectrographs have been made of the calling songs of 145 field crickets from the localities indicated in Figure 7. The recordings were made both in the laboratory and in the field, using a Magnecorder PT6A in the laboratory, and a Magnemite 610–E in the field. American Microphone Company D–33 and D–33A microphones were used in both cases, with a 24–inch parabolic reflector sometimes employed in the field. The audiospectrographs were made with a Vibralyzer (Kay Electric Company, Pinebrook, New Jersey). For further explanations of the use of this instrument and its portrayal of cricket sounds, see Borror and Reese (1952) and Alexander (1957).

Figures 9–14 show sample audiospectrographs from typical calling songs of each of the five field cricket species under consideration, and of the house cricket, *Acheta domesticus* (Linnaeus), a species introduced from Europe. The house cricket recording was made of an individual from a culture supplied to Ohio State University from the University of Missouri.

A number of analyzable characters appear in *Acheta* sounds, such as the frequency (rate of toothstrike) (individual toothstrikes are not shown in Figures 9–14), rate of pulsation (wingstroke rate), rate of chirping, regularity of chirping, number of pulses per chirp, and "musical" quality or degree of dominance of the pure frequency. Figure 15 is a scatterdiagram comparing the songs of all individuals recorded at known temperatures with respect to what is probably the most significant character, rate of wingstroke, or rate of production of sound pulses. Both laboratory and field recordings are

TABLE 1

COMPARISON OF SONG CHARACTERISTICS IN EASTERN FIELD CRICKETS

Species		*firma*	*pennsylvanica*	*vernalis*	*fultoni*	*rubens*
Number of Individuals Recorded		11	74	11	37	12
Pulses/Second (85° F.)	Range	17–19	24–29	30–33	43–50	60
	Mean	18	25	31	49	60
Chirps/Minute (85° F.)	Range	56–168	120–370	108–246	176–420	—
	Usual Number	100–120	150–240	180–200	300–360	—
Pulses per Chirp	Range	4–6	3–7	2–4	2–4	—
	Mode	4	4	3	3	—
Degree of Dominance of Pure Frequency	Rank from most "musical" to least "musical"	1	1	3	3	2
Intensity	Rank from loudest to softest	2	1	4	4	3
Frequency (Toothstrike Rate)	Rank from highest to lowest	5	4	2	3	1

included, and much of the infraspecific variation is probably due to error in measuring temperatures. Table I is a comparison of several characteristics of the calling songs of eastern field crickets.

All of the eastern field crickets sing both day and night, though *vernalis*, *fultoni*, and *rubens* do most of their singing at night. The songs of these three crickets are soft and somewhat less musical when compared to the loud, clear chirps of *pennsylvanica* and *firma*. *A. vernalis* and *fultoni* chirp more rapidly and more regularly than do *pennsylvanica* and *firma*, with *vernalis* being somewhat intermediate between the two extremes in this respect. Differences in qualities such as these are difficult to describe adequately, though they may actually be recognition characters for an observer distinguisning the songs of the different species. For example, *fultoni* chirps with considerably more regularity in southern Ohio than it does across the Appalachians in Virginia and North Carolina. This difference

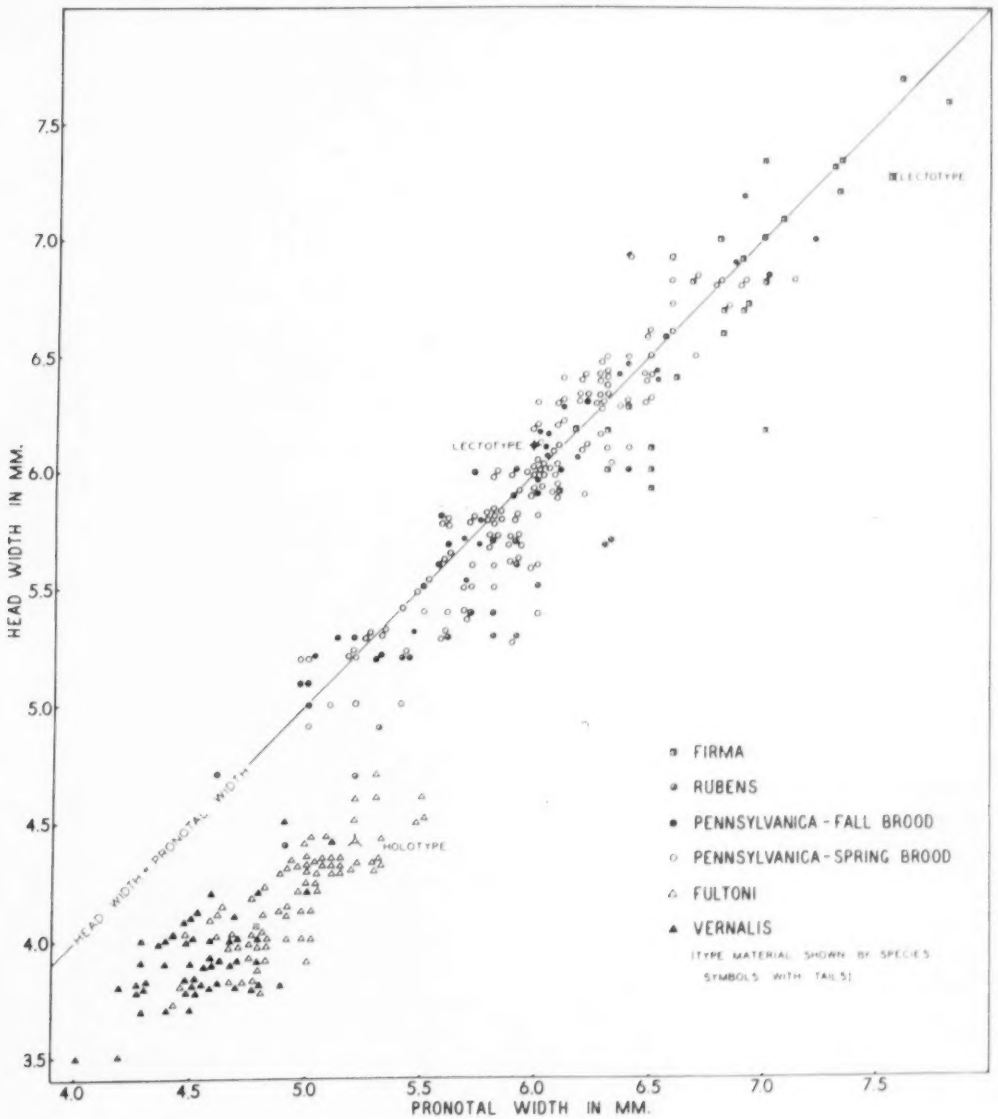

FIG. 16. The relative widths of head and pronotumin eastern field cricket males.

seems to be associated with the variation in ecology already mentioned. If uniform or rhythmical intervals in these songs are behaviorally significant, this introduction of an additional and different rhythmical element into the song of *fultoni* may be associated with its occurrence together with *pennsylvanica*, an irregular chirper. In the south, *fultoni* ordinarily occurs together with only one other species, *rubens*, which has a trilling song.

The chirping rate is not always the same in a single individual. One unmated male of *pennsylvanica* was timed singing at 120 chirps per minute shortly after molting to adulthood, and three weeks later the same male, still unmated and still caged alone, was timed singing steadily at a rate of 370 chirps per minute.

Although in certain cases other characters are more distinct and more easily used, song differences have proven better than any other single set of related characters for separating all of the *Acheta* species distinguished thus far. The recorded calling songs of all species can be separated by audiospectrographic analysis with 100 percent accuracy. Not enough courtship songs have been recorded to make comparisons, but it appears that these are more variable and less distinctive than the calling songs.

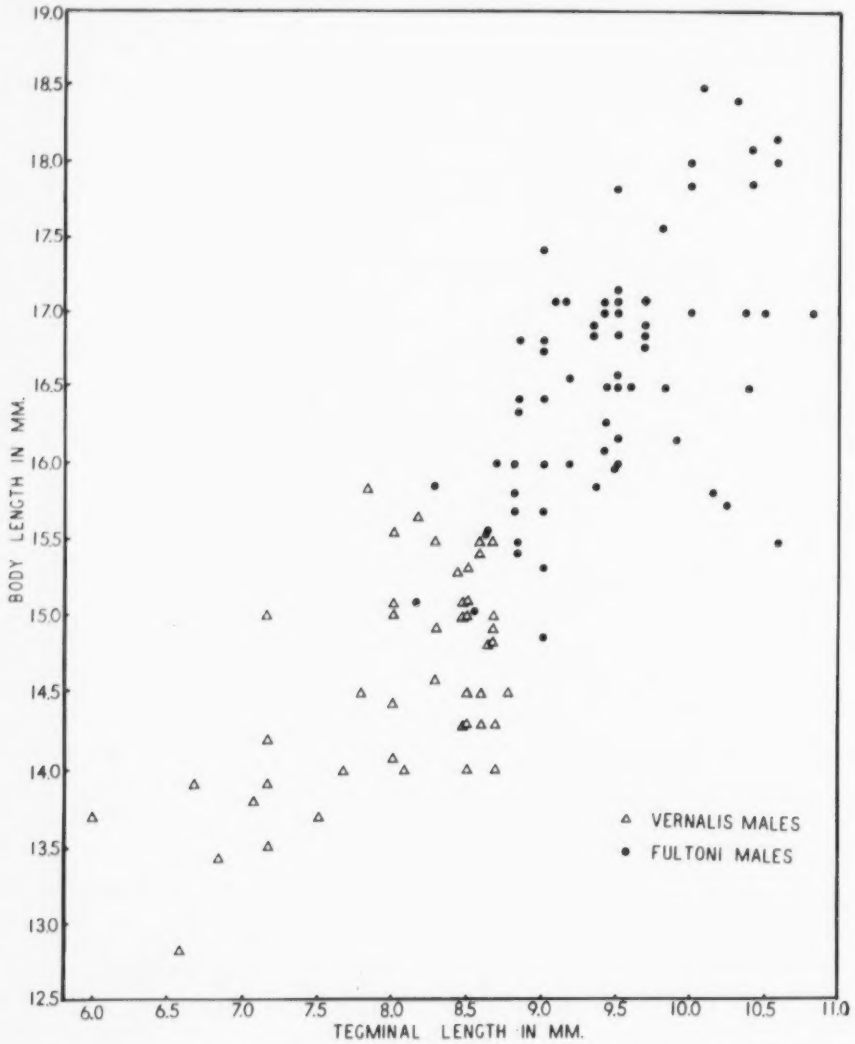

FIG. 17. The relative lengths of body and tegmina in males of *Acheta vernalis* (Blatchley) and *A. fultoni* n. sp.

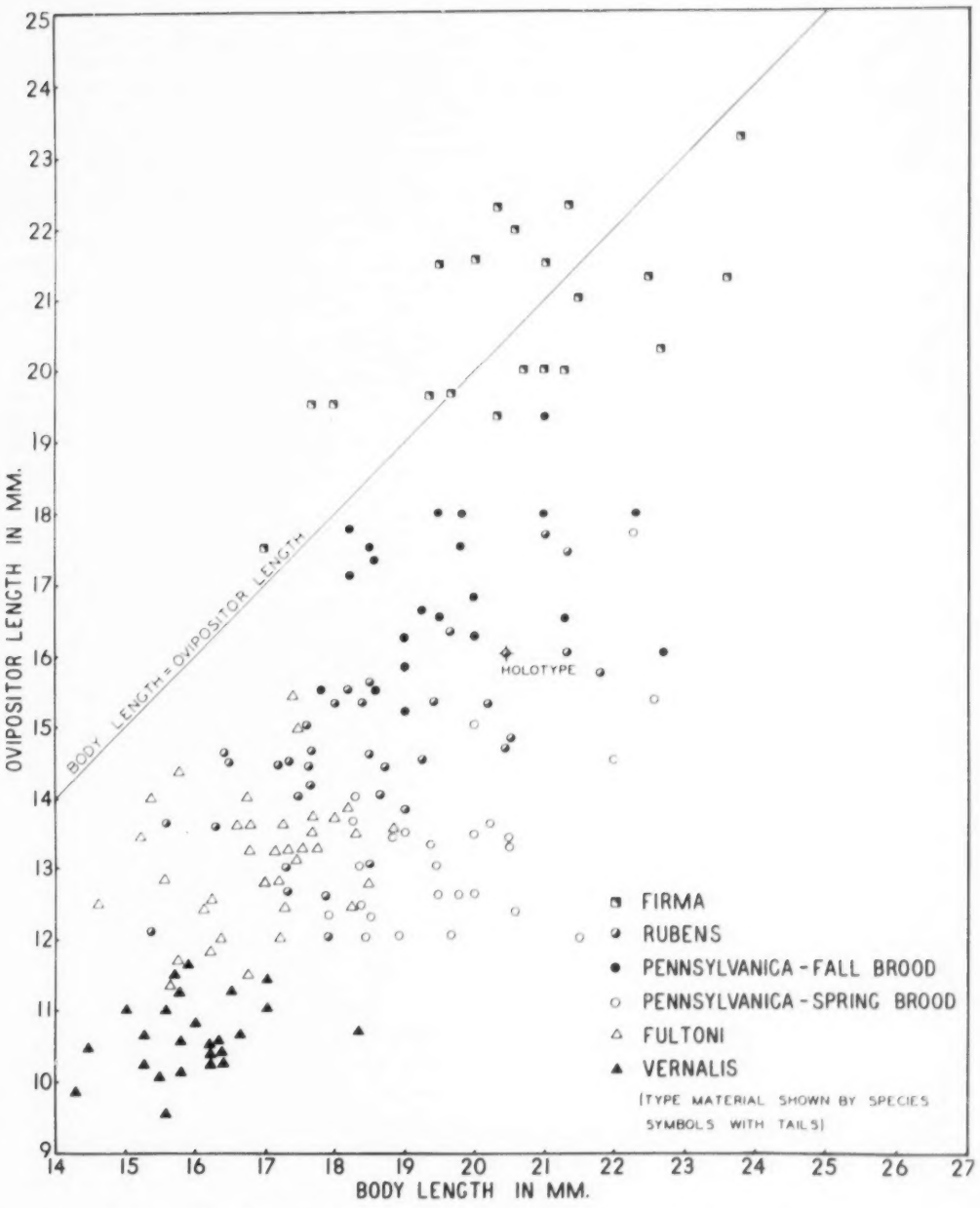

FIG. 18. The relative lengths of body and ovipositor in females of eastern field crickets.

MORPHOLOGICAL CHARACTERISTICS

The morphological characters found to be of value in this study were body proportions, size, coloration, and the length of the tegmina, wings, and ovipositor. A large number of other characters, including wing venation, structure of the male genitalia, and the number and relative lengths of the spines on the hind tibiae were examined, and none of value was discovered. No one morphological character was found which would separate all five species. Except for *vernalis*, both sexes of which are distinguishable from all the other species, most pairs of species can be distinguished in one sex only. The scatterdiagrams comparing morphological characters (figs. 16–18) are based almost entirely on the writer's collection, with type material located as designated.

Dimorphism in the length of the hind wings is exhibited by *pennsylvanica*, *firma*, and *rubens*, while only short-winged individuals of *vernalis* and *fultoni* have been collected in the field. Some individuals of *fultoni* reared on a high-protein dog food diet and under crowded conditions developed underwings slightly longer than the tegmina, but similar conditions never produced long-winged *vernalis*. Almost 100 percent of the individuals of *pennsylvanica* and *rubens* reared under these conditions develop long wings. Some authors have considered this character to be of no value since it was discovered that long-winged parents may produce short-winged offspring and short-winged parents may produce long-winged offspring. However, the ability to develop underwings functional in flight has apparently been completely lost in *vernalis* and *fultoni*.

As shown in figure 16, all species except *pennsylvanica* and *firma* have the head invariably narrower than the pronotum. In such species, the pronotum is generally widest near its posterior margin and the head appears somewhat retracted (figs. 5, 6). In *pennsylvanica* and *firma*, especially the males, the reverse is true, with the widest part of the pronotum near its anterior border, and the head as often wider than the pronotum as not, and rounded or swollen in appearance (figs. 1–3). This character of proportionate width and general conformation of the head and pronotum readily separates the males of *fultoni* and *vernalis* from those of the other species.

With respect to coloration, *vernalis* represents an extreme, being nearly always totally black. A few specimens have small reddish areas near the base of the hind femora. *A. pennsylvanica* varies from totally black specimens to specimens with light brown tegmina, pale or reddish hind femora, and pale or reddish spots on the sides of the pronotum. The pale forms are most commonly encountered in sandy areas, such as around the Great Lakes. In the writer's collection, only one out of 280 specimens of *pennsylvanica* has reddish spots on the sides of the pronotum, while 33 out of 54 specimens of *rubens* do. At present this is the best morphological character known for separating these two species. Since the ranges of these two species overlap rather narrowly, a high proportion of specimens can be separated on the basis of distribution and the coloration of the pronotum.

A. rubens and *firma* are similarly marked, generally having brown tegmina and pale or reddish hind femora, in addition to generally having reddish patterning on the sides of the pronotum. *A. fultoni* also has brown tegmina and at least partially reddish hind femora. It is separable from *vernalis* in both sexes on the basis of color alone.

A. vernalis is the smallest of the five species, with the smallest specimens seen only 12.8 mm. in body length (male). *A. firma* is the largest, with specimens in the writer's collection up to 24 mm. in body length (female). This is probably not a good indication of the upper limit in size for *firma*, but specimens positively identified as *firma* in this study are limited to those for which data other than morphological characteristics are available. Fulton (1952) includes a small number of beach crickets in the class with a body length of 26–29 mm., and Kehn and Hebard (1915) list the range of body length for American *Acheta* as from 14.0 mm. to 28.8 mm. *A. fultoni* averages slightly larger than *vernalis*, with considerable overlap, and *pennsylvanica* and *rubens* average smaller than *firma* with considerable overlap. *A. rubens* averages slightly smaller than *pennsylvanica*, and individuals from the fall brood of *pennsylvanica* average a little smaller than those from the spring brood.

As shown in figure 18, the females of *firma* are separable from those of all the other species on the basis of relative lengths of the ovipositor and body. The fall and spring broods of *pennsylvanica* are also partially separable on this basis, and the location of the syntypes of *pennsylvanica* on this diagram indicates that these were fall specimens, which is to be expected since the fall brood is generally present in larger numbers and is better represented in collections.

CROSSING EXPERIMENTS AND HYBRIDIZATION

The only evidence of hybridization of the northern crickets in the field, in spite of concentrated collecting along zones of contact and overlap, consists of one male collected in a field in southern Ohio in which both *fultoni* and *pennsylvanica* occur. This specimen, though having the narrow head and general body proportions of *fultoni*, had long black tegmina and fully developed underwings, and was larger than any *fultoni* collected. Its chirping had the typical regularity and speed of wing motion of *fultoni*, but the chirps

were four-pulse and delivered at a regular rate of about 240 per minute (80° F.). It failed to produce offspring when mated with a *pennsylvanica* female from the spring brood.

Figure 19 is a composite diagram showing the 50 crosses and 25 controls completed by Fulton (1952) and 30 additional crosses and 34 controls completed in the present study. The three successful crosses probably do not reflect a lack of reproductive isolation in the field. Adults of has been heard or collected in the field. It seems likely that song differences are of importance in this regard, although tests have not yet been performed to determine whether or not the observed differences in song are significant with respect to the behavior of the females.

In all possible types of matings between northern species, courtship, successful copulation (transference of the spermatophore), and oviposition occurred, though not in all the individual

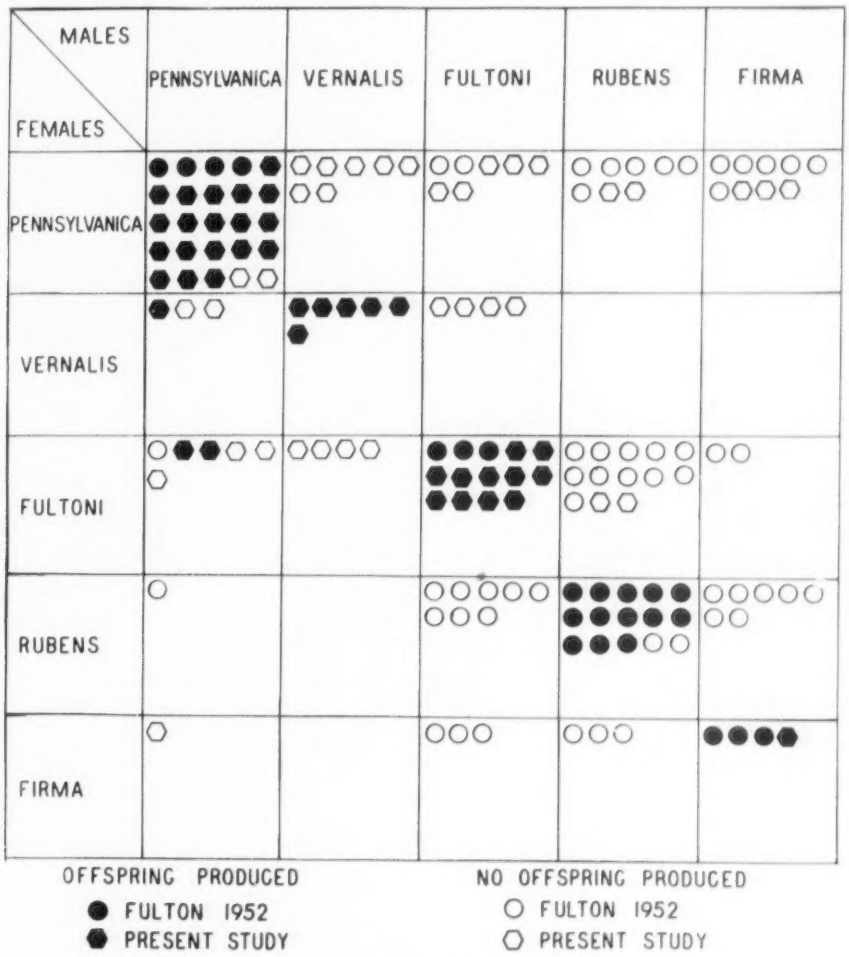

FIG. 19. Crossing experiments with eastern field crickets.

fultoni and *pennsylvanica* occur in the same places at the same time, and yet maintain their separate identities. These crosses do indicate, however, that physiological isolation is incomplete, and that the effective isolating factors in the field are probably behavioral. This suggestion is strengthened by the fact that only one presumed hybrid matings. It was noted that in matings set up at the same time these events took place sooner in the controls than in the crosses. Males in cross-matings were heard singing the calling song much more than those in the control matings. This song is generally not produced in the presence of a responsive female. Adult females in cross-

matings and virgin females caged alone often chewed their way through the cheesecloth covers of the cages and escaped, while this happened only once or twice in control cages. This increased activity could have been due to attraction to the songs of males of their own species coming from other cages in the room, or simply to a change in the physiological condition of unmated females.

PHYLOGENETIC IMPLICATIONS

Our knowledge of the distributional and other relationships of the *Achela* species is too scanty to allow any great amount of speculation as to their origin. However, certain historical aspects seem worthy of mention.

Three of the crickets under consideration have the ability to produce long-winged individuals (***pennsylvanica***, ***rubens***, ***firma***), one does not (***vernalis***), and one does so only rarely and probably imperfectly (***fultoni***). Wing reduction here is probably a secondary condition, the short-winged forms being derived from a population with the ability to form long wings. As pointed out to the writer by Dr. Fulton, in this genus as well as others, those species occurring in pioneer associations are generally able to develop long-winged individuals, while those occurring in mature or climax associations are generally not able to do so. The exact stimulus for expression of this potentiality is unknown, though it may be connected with crowding or with diet. High proportions of long-winged individuals of ***pennsylvanica*** and ***rubens*** are formed in the laboratory in crowded cultures reared on high-protein dog food. At any rate, a high degree of correlation between the capacity to produce individuals capable of flight and the degree of instability or lack of permanence in the habitat would appear to have obvious advantages.

A. ***pennsylvanica*** and ***rubens*** have both spring and fall broods, though these may differ somewhat in their relations to each other. Of the other species, ***fultoni*** and ***vernalis*** are spring crickets, and ***firma*** is a fall cricket. Both ***pennsylvanica*** and ***rubens*** exhibit considerable variation in most characters studied, while ***fultoni*** and ***vernalis***, especially, are quite uniform.

From all these comparisons one might speculate that the common ancestor of all these species was a double-brooded cricket which probably occurred in some sort of unstable or impermanent habitat and possessed the ability to produce long-winged individuals.

Settlement and clearing have undoubtedly brought about drastic changes in the relative numbers and distribution of the field crickets in eastern United States. Prior to settlement, about 95 percent of the state of Ohio was forested and five percent was prairie. Today, only about five percent of the state is forested, and the rest is chiefly involved in agriculture and perpetually in various types of grassy, weedy, or cultivated situations. Today the range of ***pennsylvanica*** is fairly continuous across Ohio, Indiana, and Illinois, while ***vernalis*** and ***fultoni*** occur only in small isolated colonies. The uniformity of these last two species across a range of 300 to 400 miles from east to west suggests, however, that their distribution was once fairly continuous. It is probable that ***vernalis*** was once the most abundant field cricket in this area, and ***fultoni*** must have occurred more or less continuously across the southern half of these states.

One of the most important consequences of finally viewing the various populations of *Achela* correctly as distinct species, is in the effect on our reconstruction of their distributional history. It is apparent that these species could not have developed as "local" adaptations to environmental conditions, with distributional relationships such as those prevailing today, and that geographic isolation on a grander scale must have once occurred. The general distributional relationships of the *Achela* species illustrate patterns very similar to those occurring between other similarly related forms in the genera *Amblycorypha*, *Neoconocephalus*, *Pterophylla*, *Oecanthus*, and *Anaxipha* (unpublished data). It seems likely that similarly related forms, perhaps also poorly defined morphologically, may exist in the non-singing Orthoptera.

KEY TO SPECIES OF *Achela* OCCURRING IN EASTERN UNITED STATES

As has already been indicated there are many cases in which preserved field crickets cannot be positively identified by morphological characters alone. One is tempted to write a key utilizing song characters, and geographical, ecological, and seasonal distribution, as well as structural features. However, these characteristics can be compared more easily by simply referring to the various illustrations. The following key is based chiefly on morphological characters, and will distinguish most preserved specimens. It should be remembered that deviations from the coloration described here may occur in specimens collected in molasses traps and cleaned by passing through various solutions, and in very old dried specimens. Thus, old pinned specimens of ***vernalis*** often develop a dull blackish red coloration, though newly pinned specimens retain the solid black color of living individuals.

1. General coloration straw or dull brownish-yellow; head and pronotum dark brown, mottled with paler areas; head with an irregular dark, transverse bar extending between the eyes near their dorsal border, narrower light bars in front of and behind this bar. . . . . **domesticus**

1'. General coloration ranging from solid black to dark brown, tending toward reddish in paler specimens rather than toward straw-color; head and pronotum black, with or without pale markings; head without transverse bars. 2

2(1'). Pronotum of male widest near its posterior border; head of male definitely narrower than

pronotum, presenting a retracted appearance, not exceeding 4.7 mm. in width; body length 12.8–19 mm.; ovipositor length 9.5–15.5 mm.; always micropterous in both sexes 3

2'. Pronotum of male usually widest near or anterior to its middle; head of male as often wider than pronotum as not, presenting a swollen globose appearance, almost always over 4.7 mm. in width; body length 15–29 mm. or more; ovipositor length 12–24 mm. or more; macropterous or micropterous in either sex 4

3(2). Coloration solid black, rarely a dash of reddish on the inside or outside of base of hind femora . . . . . **vernalis**

3'. Tegmina always brown; hind femora usually reddish or pale, at least on basal third; tibiae pale . . . . . **fultoni**

4(2'). Found along the Atlantic Coast (and probably the Gulf Coast), and in sandy areas in the Southeast; body usually over 18 mm. in length; ovipositor 19–23 mm. or more in length and as often longer than the body as not . . . . . **firma**

4'. Found in fields and grassy areas throughout eastern United States, including the areas described above; body rarely over 23 mm. in length; ovipositor rarely over 18 mm. in length, and always shorter than body . . 5

5(4'). Pronotum usually with reddish or light-colored spots on sides; southern crickets occurring northward only into the Appalachians and to the southern tips of Illinois and Missouri **rubens**

5'. Pronotum almost always lacking light spots on sides, except in crickets collected in northern sandy areas, such as around the Great Lakes; northern crickets extending southward to just below the Appalachians . . . . . **pennsylvanica**

SUMMARY

The singing Tettigoniidae and Gryllidae of eastern United States contain a number of species which have not received formal recognition by taxonomists because morphological characters are not known by which preserved specimens can be distinguished. Among these are five species of *Acheta*, four of which were shown by Fulton (1952) to be non-interbreeding populations in North Carolina. All but one pair of these species can now be separated, in at least one sex, on the basis of morphological characters whose value had previously been obscured by (1) attempts to deal with the whole genus at once, and (2) studies based on morphological relationships alone. All five species can be separated on the basis of song differences revealed by audio-spectrographic analysis.

Acheta pennsylvanica (Burmeister) is the most abundant species in northeastern United States. It occurs in grassy and weedy areas, has a chirping song, and has two single-brooded populations, one adult in spring and one adult in fall. *A. rubens* (Scudder) is the most abundant species in southeastern United States. It also occurs in grassy and weedy areas, but it consists of a single, double-brooded population, producing both spring and fall adults, and it has a trilling song. *A. vernalis* (Blatchley) and *A. fultoni* n. sp. are chirping crickets which occur in leaf litter in woods in northeastern and southeastern United States, respectively. *A. firma* (Scudder) is a chirping fall cricket which occurs along the Atlantic coast south from Delaware, and inland in sand hills in North Carolina. North-south variations in ecology and song occur in *A. fultoni*, and certain variations in life history occur in *A. pennsylvanica*.

It is probable that at least one eastern species and several western species of *Acheta* in the United State are as yet unrecognized.

ACKNOWLEDGMENTS

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PAPER CHROMATOGRAPHY STUDIES OF THE ANOPHELES MACULIPENNIS COMPLEX IN CALIFORNIA (DIPTERA: CULICIDAE)[1]

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Taxonomic studies of the *Anopheles maculipennis* complex in California have been based to a large extent on the presence or absence of wing markings of the adult. This is evident from the work by Aitkin (1945) in which the light colored, coppery to silver-white mark at the apex of the wing in *Anopheles occidentalis* Dyar and Knab is used as the primary criterion for separation from *Anopheles freeborni* Aitkin which has unicolorous, dark wing scales.

Ecologically, *A. occidentalis* is a cool-climate species breeding along the coast and never extending very far inland, while *A. freeborni* is found predominately in certain warm, interior valleys of California. Typical *occidentalis* populations extend along the coast from roughly Pismo Beach northward to the Oregon border. From Pismo Beach southward to the Mexican border, coastal populations of the *maculipennis* complex occur which on the basis of the presence or

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absence of the wing mark have been reported as either *occidentalis* or *freeborni* or even both from the same locality (Freeborn and Bohart 1951). These populations may actually all be *occidentalis*, some individuals of which lack the typical mark at the wing apex. The difference in ecology of these two species lends support to this hypothesis. On this basis, the morphological approach has not been entirely satisfactory for distinguishing between all populations of *occidentalis* and *freeborni*.

Paper chromatography studies of similar taxonomic problems have shown promise in untangling such species complexes (Micks 1954), and it would appear likely that this technique could be applied to the California anopheline complex. It is reasonable to assume that differences in the biochemical makeup of these two species would be sufficiently distinct to reveal consistent differences in the ninhydrin-positive patterns formed by protein constituents as revealed by one-dimensional paper chromatography, and that these differences could be used as criteria in taxonomic differentiation.

METHODS AND MATERIALS

Populations of *Anopheles occidentalis* were studied from the following areas: Southern form: Agoura (Santa Monica Mountains, Los Angeles County), Sand Canyon (Orange County), and Jamacha Junction (San Diego County). Northern form: Pismo Beach (San Luis Obispo County), and Davenport (Santa Cruz County). *Anopheles freeborni* populations were sampled from Buttonwillow (Kern County),[3] Resting Springs (Inyo County), and Maxwell (Colusa County).

Material for the preparation of chromatograms was obtained by two methods. Either adult gravid females were collected under culverts and low bridges by means of an aspirator and brought into the laboratory for progeny rearings, or in situations where adult females were unobtainable, larvae were collected from the breeding site and reared in the laboratory to the adult stage. All collections were made during the period May to September, 1956.

Field collected females were usually placed in a rearing cage 36 x 15 x 15 inches with a micarta top and bottom, wood framing, and 16 mesh plastic screening on the back and sides. The front of the cage consisted of an acetate sheet on the upper portion extending from the top downward 25 inches, the remainder of the front being occupied by a muslin access sleeve about 30 inches long. Food was provided by means of a piece of cellucotton placed in a 9 cm. petri dish and saturated with tap water. Raisins were placed on top of the wet cellucotton, usually about six to eight being placed in each dish. The mosquitoes appeared to feed not only on the moist raisins but on the juice that seeped into the cellucotton as well. No blood meal was provided since the condition of the female specimens indicated that blood had been obtained in the field.

The oviposition site consisted of a plastic cereal dish about one quarter full of distilled water with a filter paper funnel made from a disk 27 cm. in diameter which rested in the dish with the bottom part of the taper immersed in the water. The funnel was made by cutting out one-fourth of the disk in a pie shape and stapling the cut edges together. The bottom orifice of the funnel was about one inch in diameter. Eggs were deposited on the moist filter paper, near the water line.

The other method of collecting eggs, consisted of isolating gravid females in individual oviposition tubes which consisted of a test tube 15 cm. long x 1.5 cm. in diameter with a strip of filter paper 13 cm. long x 1.2 cm. wide inserted into the tube and moistened with tap water; the tube was plugged with a piece of cellucotton. The close confinement of these conditions was believed to force oviposition, thus making eggs available faster for rearing, although this method produced a high degree of mortality. Frequently viable eggs were found in tubes containing dead mosquitoes, indicating that the female had laid eggs just prior to death. The oviposition tubes were inspected at 24 hour intervals.

Eggs were collected from the filter paper cones or strips and placed in 30 x 20 x 5 cm. enamel pans containing 1500 ml. tap water which was constantly aerated by means of a small air compression pump. The air was distributed to the series of rearing pans (10 maximum) from the main air line by means of ¼ inch glass tees inserted into the main line and leading to each individual pan by means of an eight inch piece of ¼ inch rubber tubing. Air pressure to each pan was regulated with a Hoffman tubing clamp placed on the ¼ inch tubing leading into the pan from the main air line. The air outlet into the water consisted of a medicine dropper inserted in the end of the rubber tubing. Aeration of the water prevented stagnation and the development of organisms deleterious to the larvae.

Larval food consisted of equal parts of ground laboratory chow[4] and dried brewer's yeast. The larvae were fed by lightly sprinkling the mixture on top of the water from a salt-shaker type container until the food stopped skating on the water surface.

All rearing was carried out at room temperature; therefore it was impossible to get exacting data on the life history of these species under laboratory conditions. Nevertheless, the following observa-

[3]Supplied by R. E. Bellamy, U.S. Public Health Service, Encephalitis Laboratory, Bakersfield, Calif.

[4]Ralston Purina Company, St. Louis, Missouri; Purina Laboratory Chow (micro-mixed).

tions were made. *Anopheles occidentalis* southern form (Sand Canyon): egg stage, 4 days; larval stage, 15 to 17 days; pupal stage, 3 days. *Anopheles freeborni* (Buttonwillow): egg stage, 3 days; larval stage, 14 days; pupal stage, 3 days. (Maxwell): egg stage, 3 days; larval stage, 16 days; pupal stage, 3 days.

PREPARATION OF CHROMATOGRAMS

Most chromatograms were prepared from newly emerged, unfed adult mosquitoes obtained either by laboratory rearing from eggs, or from field collected larvae reared through to the adult stage. A few chromatograms were prepared from field collected females that had not yet obtained a blood meal, and compared with chromatograms of laboratory reared specimens from the same locality with no differences in the results. This indicates that ecological differences which existed between laboratory reared specimens and field collected specimens had no effect on the chromatographic pattern produced.

In preparing paper chromatograms of mosquito adults, fresh tissue techniques were always employed. Living mosquitoes confined in shell vials were immobilized by the fumes from five or six drops of carbon tetrachloride placed on a cellucotton plug. For localities with little material available, smashes were made on the papers by placing the immobilized mosquito on a mark centered 13 mm. from the bottom edge of a Whatman 1 filter paper strip 180 mm. long x 23 mm. wide at the top, tapered to 17 mm. wide at the bottom; the mosquito was then crushed on the paper with a glass rod. Extraneous parts such as the head, wings, and legs were carefully picked away with forceps leaving material from the thorax and abdomen. This proved to be as satisfactory as cutting off extraneous parts first with dissecting scissors, and was much more rapid.

For localities where abundant material was available, especially from laboratory rearings, fresh tissue homogenates were prepared. The homogenates were prepared by placing 50 immobilized mosquitoes of either sex in a Potter-Elvejem homogenizer tube and macerating in 1 ml. of distilled water. The homogenate was centrifuged at 1,000 times gravity for five minutes. After centrifugation the supernatant liquid was strained through two layers of cheesecloth and collected in a small beaker. The strained liquid was grayish-white.

The extract was applied to the chromatograms by means of a one-fourth cubic centimeter tuberculin syringe fitted with a 27 gauge needle with the point ground off flat; the needle was bent down at 90 degrees from the base. The syringe was held horizontally in a wooden block to which a modified micrometer was mounted that was arranged to advance the plunger so that 1.0 cubic millimeter (approximately 1 lambda) of liquid was delivered with each five-thousandths inch turn of the barrel. This type of apparatus has been used extensively for applying measured drops of insecticides to house flies (March and Metcalf, 1949).

Each chromatogram strip was prepared by spotting the extract on a pencil mark centered 13 mm. from the bottom edge of a filter paper strip of the same dimensions as previously described for the smash method. Each strip was spotted in the same area six times, allowing the paper to dry between each spotting. After drying completely at room temperature following the final application of material, the papers were suspended in test tubes 195 mm. long x 25 mm. in diameter, by wedging the wider top portion against the sides of the tube, allowing the remaining, narrower four-fifths of the strip to hang freely in the tube with the bottom end emersed in the solvent. Each tube contained 2 ml. of *n*-propanol-NH_3 solvent (Buzzati-Traverso, 1953) and was plugged with a cork. The strips were developed for approximately 4 hours at 10° C., after which the papers were removed, the solvent front marked, and the strips hung to dry at room temperature.

The ninhydrin-positive pattern was revealed by spraying the strips with a 0.2 percent ninhydrin solution in 95 percent ethanol to which 5 percent 2, 4, 6-collidine was added just before using (Buzzati-Traverso and Rechnitzer, 1953). The spots appearing on the chromatograms were blue, violet, gray-blue or pink depending on the nature of the constituent.

In general, homogenate preparations tended to give a sharper separation of the constituents than the smash method; frequently trailing was evident on the smash preparations, but this did not introduce an element of uncertainty in delimiting the areas containing the constituents.

Although in taxonomic studies comparisons are frequently made on the basis of a series of 20 specimens for each category examined, enough material was not available to prepare 20 chromatograms of each population for this study. The results are based on comparisons of the following number of one-dimensional smash chromatograms for each population: Agoura—male 2, female 1; Sand Canyon—male 3, female 7; Jamacha Junction—male 3, female 2; Pismo Beach—male 2, female 3; Davenport—male 0, female 1; Maxwell—male 4, female 4; Resting Springs—male 5, female 5; Buttonwillow—male 4, female 2.

Six chromatograms of each sex were prepared from homogenates of the Sand Canyon and Buttonwillow populations with the same results as the smash method.

EXPERIMENTAL RESULTS

The average R_f values obtained for the various populations are given in Tables I and II.

TABLE I

AVERAGE R_f VALUES OF NINHYDRIN-POSITIVE PATTERNS OF FEMALE *Anopheles occidentalis* AND *A. freeborni*

LOCALITY AND SPECIES	VIOLET	VIOLET	VIOLET	BLUE	GRAY-BLUE	VIOLET	YELLOW	PINK
occidentalis (northern form)								
Davenport		0 10	0 22		0 34		0 45	0 53
Pismo Beach		0 13	0 22		0 34		0 45	
occidentalis (southern form)								
Agoura				0 24		0 36	0 44	
Sand Canyon				0 29		0 39	0 46	
Jamacha junction			0 21	0 27		0 38	0 49	
freeborni								
Maxwell	0 06	0 12	0 20	0 27	0 34		0 43	0 53
Buttonwillow	0 09	0 14	0 19	0 24	0 30	0 38	0 45	0 53
Resting Springs	0 09	0 13	0 20	0 25	0 30	0 35	0 43	0 52

TABLE II

AVERAGE R_f VALUES OF NINHYDRIN-POSITIVE PATTERNS OF MALE *Anopheles occidentalis* AND *A. freeborni*

LOCALITY AND SPECIES	VIOLET	VIOLET	VIOLET	BLUE	GRAY-BLUE	VIOLET	YELLOW	PINK
occidentalis (northern form)								
Pismo Beach		0 11	0 22		0 34		0 48	0 53
occidentalis (southern form)								
Agoura			0 24		0 32	0 40	0 45	
Sand Canyon		0 16	0 22	0 26	0 33		0 49	
Jamacha Junction				0 26	0 35	0 42	0 49	
freeborni								
Maxwell	0 08	0 14	0 19	0 25	0 30	0 36	0 45	
Buttonwillow	0 09	0 16	0 21	0 28		0 35	0 44	0 52
Resting Springs		0 13	0 20	0 27	0 34	0 39	0 43	0 55

DISCUSSION

The data presented in Tables I and II disclose that typical northern *occidentalis* had chromatographic patterns consisting of four or five spots; the pattern of southern coastal forms of the *maculipennis* complex consisted of three to five spots, while the pattern of *freeborni* populations consisted of seven or eight spots.

From these preliminary studies it appears that the southern coastal form of the *maculipennis* complex is closer in pattern to the typical northern form of *occidentalis* than it is to *freeborni*. This indicates that the southern *occidentalis* populations have probably lost the pale mark at the wing apex and therefore resemble the unicolorous winged *freeborni*. More studies of this nature and with more material than was available for the present study will be necessary before this view can be substantiated. However, the results obtained indicate the possibilities of using paper chromatography as an approach to clarifying the *Anopheles maculipennis* complex in California.

A difference in pattern between the male and female of the same population from the same locality is evident in some cases with the male having one or two additional spots in *occidentalis*. The reverse appears to hold with *freeborni*, the female in some instances having more spots than the male. Also differences in the number of spots are evident in samples of the same species from different localities. Despite this seeming variation between populations of the same species, in the overall pattern there is consistency when *occidentalis* is compared with *freeborni*.

SUMMARY

Adult characters of the *Anopheles maculipennis* complex in California have not been entirely satisfactory in distinguishing some populations of *Anopheles occidentalis* from *A. freeborni*. *A. occidentalis* is typically a coastal species with a coppery to silvery mark at the wing apex in populations from Pismo Beach northward, while populations from Pismo Beach southward often lack the wing mark or have an indistinct one, thus resembling *A. freeborni*.

Procedures for collecting and rearing these two mosquito species in the laboratory are discussed, as well as methods of preparing chromatograms for comparison.

Comparisons of fresh-tissue, one-dimensional, ascending chromatograms revealed that the ninhydrin-positive patterns formed by protein constituents from the northern and southern *occidentalis* populations showed closer affinity to

one another than either did to the *freeborni* pattern. The northern *occidentalis* chromatograms contained four or five ninhydrin-positive spots; the southern *occidentalis* contained three to five spots, while the *freeborni* chromatograms contained seven or eight spots.

A difference in pattern was noted between male and female populations from the same locality, in most instances, as well as differences between sample populations of the same species from different areas. These differences did not affect the overall pattern for comparison of the two species.

The preliminary results indicate that paper chromatography shows promise as another means of distinguishing between *occidentalis* populations and *freeborni* populations.

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A COMPARISON OF FIVE METHODS OF SAMPLING ALFALFA FIELDS FOR ARTHROPOD POPULATIONS[1]

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In 1954 a study was undertaken to determine the effects of several insecticides on the insect, spider, and mite populations in an alfalfa field. One of the first problems was to develop a standard method of sampling which would have a minimum amount of experimental error. A review of literature showed that several methods had been used by other workers. Five methods were tested and some were modified. This paper is a report on these tests, most of which were made in 1955.

METHODS USED IN THIS STUDY

The sweep net.—Delong (1932), Gray and Treloar (1933), Beall (1935), Romney (1945), and Whittaker (1952), experimented with and evaluated the sweep net method of sampling for insect populations in various types of habitats from low-growing crops such as alfalfa to trees and shrubs. Their conclusions varied from unfavorable (DeLong, Gray and Treloar) to favorable provided the catch was correlated with temperature and wind velocity taken at the time sweeps were made (Romney).

In this study the sweep net had a diameter of 13 inches and a handle length of 19 inches. Only one pass was made with the net while the next and succeeding strokes were always made in parts of the field not previously disturbed by the net. In short sparse growths most of the plants were included in a single stroke but as the plants grew taller, less and less of them were hit with the net. Five samples were taken per field, each one in a different area and at each point from 10 to 50 sweeps constituted a sample. The insects and other arthropods collected were immobilized at once. This was accomplished by pouring a few drops of carbon tetrachloride on a piece of absorbent towelling paper at the bottom of a cardboard container. The net was then partially everted so that the insects and other animals in the net were inside the container. Finally a few more drops of the fumigant were added to the net. At least one minute was allowed to elapse before emptying the contents of the net into the container. Each collection was either examined within 24 hours or preserved in 80 percent alcohol for later examination.

The proportion of insects resting on the plants which were caught by the net was determined by the following tests: First, the number of tillers included in one stroke of the net was found to range from 124 to 177 with an average of 155 in those parts of the field where the stand was perfect. This meant in an average stand of alfalfa, having few or no skips in the rows, that 19,375 tillers were sampled for each 125 strokes of the net. Since, however, the stand was not perfect in areas of this field which were sampled, probably 15,000 tillers is a conservative estimate and this number has been used in our compilations.

Secondly, one-row plots were set up having the approximate number of tillers that would be swept with one stroke of the net. Such plots were located in various parts of the field and were prepared for sampling by the simple method of

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cutting down all of the other plants on both sides of each row as well as at both ends. This was done the day before the samples were taken. The procedure followed in making the tests was to make one pass with the net so as to include all of the plants in the row. The insects and spiders collected in this one net stroke were then removed from the net and preserved according to the procedure previously described, following which the row was repeatedly swept until all insects remaining on the plants were taken. These tests were made in an alfalfa field gone to seed and in a plot where the growth was rank and continuing. Except for thrips and alfalfa aphids, from 44.2 to 71.4 percent of all the insects and spiders resting on the plants were taken by the first net stroke.

Plant clippings.—Shortly after the sweeps were made, five tillers were clipped at random from each of five points, also selected at random, in the field. Each clipping was immediately placed in a wide-mouth one-quart fruit canning jar to which a little carbon tetrachloride had been added. Thus 25 clippings constituted a sample for each date of collection. In tall alfalfa plants only the upper portion of a tiller was taken. The insects were separated from the clipping by placing the plants in a flask of 60% alcohol, and thoroughly shaking the mixture. Two or three rinses were necessary to remove all insects and spiders.

Berlese funnels.—A modification of the Berlese funnel as first built by Berlese (1905) was used to process surface trash and soil to separate the

TABLE I

NUMBER OF SPOTTED ALFALFA APHIDS AND THRIPS COLLECTED IN 125 SWEEPS WITH ESTIMATED PERCENTAGES OF POSSIBLE TOTALS PRESENT ON THE PLANTS AS DETERMINED BY PLANT CLIPPING COUNTS, STILLWATER, OKLAHOMA, 1955

DATES	THEORETICAL NUMBER OF APHIDS AND THRIPS PER 15,000 TILLERS BASED UPON PLANT CLIPPING COUNTS		ACTUAL NUMBER OF APHIDS AND THRIPS COLLECTED FROM 15,000 TILLERS BY 125 SWEEPS		PERCENT TAKEN BY SWEEPS		TEMP. °F.	WIND MPH.
	Aphids	Thrips	Aphids	Thrips	Aphids	Thrips		
June 15–17	27,000	104,000	551	8	2	0.007	90	7½
June 25–27	38,400	105,600	1,078	64	2.8	0.06	83	5
July 11–13	40,200	94,200	2,980	73	7.4	0.08	91	5
July 25–27	0	40,800	755	14	?	0.03	86	0
Aug. 22–24	7,200	49,200	474	64	6.5	0.13	84	5
Sept. 6–8	1,200	105,000	269	101	22.4	0.96	77	5
Sept. 21–23	7,200	28,800	591	23	8.2	0.08	95	4½
Oct. 10–12	1,800	20,400	118	20	6.5	0.098	78	5
Oct. 26–28	8,400	54,000	845	10	10.0	0.02	81	12

TABLE II

COMPARATIVE NUMBERS OF COLLEMBOLA[a] AND MITES TAKEN IN CLIPPINGS, SWEEPS, AND PIT TRAPS, STILLWATER, OKLAHOMA, 1955

DATES	NUMBER PER 25 CLIPPINGS		NUMBER PER 125 SWEEPS		NUMBER PER 4 PIT TRAPS	
	Collembola	Mites	Collembola	Mites	Collembola	Mites
June 15–17	0	2	0	0	38	52
June 25–27	0	0	0	0	140	92
July 11–13	0	0	2	0	45	81
July 25–27	0	1	2	0	169	93
Aug. 22–24	0	0	0	0	117	80
Sept. 6–8	0	3	0	34	68	61
Sept. 21–23	0	8	1	1	75	47
Oct. 10–13	0	0	2	0	145	23
Oct. 26–28	0	0	0	0	19	30
Totals	0	14	7	35	816	559

[a] *Pseudosinella violenta* Fols., *Achorutes humi* Fols., *Entomobrya purpurascens* Pack., *Entomobrya multifasciata* Tull., *Lepidocyrtus cyaneus* var. *anescens* Guthrie, *Lepidocyrtus cyaneus* var. *cinereus* Fols., *Lepidocyrtus cyaneus* Tull., *Lepidocyrtus unifasciatus* James, *Isotoma viridis* var. *delta* MacGil., *Depranura sabulicola* Mills, *Folsomia elongata* MacGill., *Sminthurides pumilis* Kraus.

contained insects. The one used in this experiment was a metal funnel 11 inches deep attached to a cylinder with a diameter of 11 inches and a depth of 14 inches. The lower opening was attached to the screw top of a glass fruit preserving jar. The latter, screwed to the funnel, was used to trap the arthropods which were forced out of the sample by the heat generated by a 200-watt electric light bulb. The socket of this bulb was soldered to the center of a metal cover which fitted tightly over the top end of the cylinder. The sample, which was composed of surface soil, trash, and plant roots from one-half square foot area, was supported on a 14-mesh wire screen at the bottom of the cylinder.

Pit traps.—Barnes (1953) and Fichter (1954) used pit traps to measure surface inhabiting arthropod populations. In our study one-half pint wide-mouth glass fruit preserving jars were used in groups of four at approximately three-foot intervals. Each jar was charged with about one inch of water to which a teaspoonful of 40 percent formalin and a pinch of detergent had been added. The period of exposure was 48 hours.

Henderson sampler.—A fork-type sampling device, designed by Henderson (1956), was used concurrently with sweeping as a means of comparing the two methods of sampling for the spotted alfalfa aphid, *Therioaphis maculata* (Buck.). This device consists of a fork-like hand tool which holds four narrow strips of window glass covered with an adhesive material to hold the trapped aphids in place for later examination. The fork is placed in position on the ground beneath the plants and the plants are shaken to dislodge the aphids.

RESULTS

Effectiveness of the sweep net in collecting aphids and thrips.—Assuming that most of the aphids and thrips remained on the plants until the clipping was transferred to the jar gives a base for calculating the percentage taken by the sweep net, since the average number of tillers taken in by 125 sweeps is given as 15,000. In this calculation, however, two things must be taken into consideration: first, in many samples only the upper part of the tiller was clipped; and secondly, it is well known that some of the spotted alfalfa aphids drop from the plant upon the slightest disturbance. Therefore, the figures given in Table I must be taken as the *minimum* number of thrips and spotted alfalfa aphids calculated to be present on 15,000 tillers. It is seen that the sweep net collected from 2 to 22.4 percent of the minimum number of aphids present on the tillers, except for the period of July 25–27 when none were taken. It is evident that usually less than 10 percent of the aphids are taken from the plants in the net, and considerably less than one percent of the thrips in most cases. The discrepancy shown in the July 25–27 counts can be accounted for by the fact that the sweep net covered much more of the field being sampled than the spot clipping method.

Mite and Collembola populations best sampled by pit traps.—Table II shows the numbers of Collembola and mites collected by sweeps, clippings and pit traps. It appears that most of these small arthropods are inhabitants of the soil surface in an alfalfa field and that comparatively few occur on plants. In the case of mites, the few collected in sweeping were mostly plant feeders. Collembola were occasionally collected by sweeping, and one species, *Deuterosmynthurus* sp.[2] was swept from alfalfa plants in large numbers on certain dates. However, sampling by pit traps was necessary to record the mite and Collembola populations found in alfalfa fields.

TABLE III

SUMMARY OF NINE 48-HOUR PIT TRAP COLLECTIONS, JUNE 15–OCTOBER 28, 1956, STILLWATER, OKLAHOMA

ITEM	TOTAL COLLECTED	MAXIMUM COLLECTED DURING 48 HOUR
Coleoptera (misc.)	52	23
Carabidae (adults)	36	13
Carabidae (larvae)	32	13
Cicindellidae	15	6
Monotomidae	52	19
Meloidae	315	255
Hymenoptera (misc.)	91	32
Formicidae	228	68
Diptera (misc.)	22	11
Chloropidae	84	18
Phoridae	39	20
Lycoridae	86	35
Lepidoptera (misc.)	5	2
Hemiptera (misc.)	41	35
Homoptera (misc.)	16	5
T. maculata	196	121
Orthoptera (misc.)	19	10
Collembola (misc.)	816	169
Thysanoptera	10	5
Acarina (mites)	559	93
Araneida	165	7

Other species taken in pit traps, rarely in sweeping.—As might be expected, beetles of the family Carabidae were commonly collected in the pit traps, but most of them rarely, if ever, by sweeping. In two years of collecting we obtained only one species regularly by sweeping, namely *Lebia viridis* Say. In sixteen 48-hour periods of collection Carabidae were taken on 13 dates totaling 189 adult specimens (Table III). Of these, the following three have been identified: *Poecilus chalcites* Say, *Scarites substriatus* Hald., and *S. subterraneus* Fab. Rarely was a *Lycosa* spider taken in sweeping, but quite a number were found in the pit traps. The same was true for two species of Cicindellidae, namely, *Cicindella punctulata* Oliv., and *Taracha virginica* L.

Other records from pit traps.—Ants of several

[2]Found to be a new species by D. L. Wray.

TABLE IV

List of Identified Species Collected by Sweeping Alfalfa Fields During 1955 Based on 100 Sweeps per Date

Species	6/15	6/27	7/13	7/27	8/24	9/7	9/21	10/10	10/26	Totals
COLEOPTERA										
Collops 4-maculatus	1	1	6	18	8	9	3	0	0	46
Scymnus natchezianus	1	0	0	1	1	0	1	0	2	6
Diabrotica 12-punctata howardi	2	1	13	8	3	164	32	8	16	247
Lebia viridis	1	0	1	1	0	2	2	4	2	13
Haltica foliacea	1	0	5	1	3	1	0	2	0	13
Collops balteatus	0	0	1	1	1	7	1	0	0	11
Epicauta lemniscata	0	0	0	74	0	48	1	0	0	123
Epicauta unicolor	0	0	0	4	7	0	0	0	0	11
Hippodamia convergens	19	29	209	101	33	12	2	4	0	409
ORTHOPTERA										
Melanoplus mexicanus	1	0	7	3	0	1	0	1	0	13
Oecanthus nigricornis	2	1	29	32	2	5	4	2	0	77
HYMENOPTERA										
Chelonus texanus	0	1	9	0	3	0	2	0	0	15
Aphidius testaceipes	0	17	0	0	0	0	0	0	0	17
Cremnops vulgaris	0	5	0	1	0	2	1	0	0	9
Diplazon laetatorius	0	3	9	0	0	0	0	0	0	12
Apis mellifera	0	0	9	0	11	1	0	0	0	21
Agathis gibbosus	0	0	77	20	3	14	482	64	26	686
Liodontomerus perplexus	0	0	4	5	1	0	102	248	330	690
DIPTERA										
Thaumatomyia glabra	1	3	196	40	56	155	61	0	14	526
Hylemya cilicrura	5	9	0	0	0	0	0	14	14	42
HEMIPTERA										
Psallus seriatus	1	1	20	7	22	8	6	0	0	65
Lygus lineolaris	29	222	500	458	13	26	18	42	72	1080
Orius insidiosus; O. tristicolor	17	122	474	189	114	197	11	6	12	1142
Thyanta pallido-virens accerra	2	0	0	13	5	18	10	16	18	82
Nabis species	2	39	39	24	22	25	4	4	12	171
Nysius raphanus	1	4	16	173	27	0	1	6	0	228
Nysius californicus	0	0	14	6	0	2	1	0	0	23
Jalysus wickhami	1	1	5	4	6	0	0	2	0	19
Geocoris pallens; G. punctipes	2	0	5	2	21	1	0	0	0	31
Liorhyssus hyalinus	0	0	10	20	9	1	0	0	0	40
Sinea diadema	0	0	5	4	0	8	6	8	0	31
Aceratagallia uhleri	7	14	14	21	5	23	8	0	14	106
Empoasca fabae	52	156	88	27	62	135	10	34	4	568
Macrosteles fascifrons	1	4	2	1	8	30	1	42	16	105
Vanduzea triguttata	0	0	0	0	7	5	5	2	2	21
Spissistilus festinus	1	4	28	25	8	24	30	26	26	172
Nesosteles species	2	3	5	11	3	13	1	102	2	142
Macrosiphum pisi	106	523	0	0	0	0	0	0	0	629
Therioaphis maculata	441	862	2384	604	379	215	473	94	676	6128
LEPIDOPTERA										
Caenurgina erechtea	1	2	0	1	6	4	1	4	2	21
Euerythema species	2	0	1	0	12	11	1	0	2	29
Loxostege similalis	1	0	1	1	11	2	2	0	0	18
THYSANOPTERA										
Frankliniella occidentalis	6	51	58	11	51	81	18	16	8	300

species were usually present in the collections and, at certain times of the year, they were also taken in sweeps. Adults and larvae of the convergent lady beetle, ***Hippodamia convergens*** Guer., were also collected and in one year an elaterid beetle, ***Monocrepidius vespertinus*** (Fab.) was taken in large numbers. Both of these species were also taken in large numbers by sweeping. The three-striped blister beetle, ***Epicauta lemniscata*** Fab. is another example of a species commonly taken by both methods. The spotted alfalfa aphid was frequently found, and in one 48-hour period more of these aphids were collected in traps than in sweeping.

One of the surprising developments of the pit trap collections was that Diptera and Hymenoptera were frequently represented by several species. These were usually very small species such as the Chloropidae and Phoridae in the Diptera and representitives of several families of parasitic Hymenoptera not as yet identified. Among the small beetles were the families Monotomidae and Pselaphidae. In the case of Diptera the specimens were preserved in much better condition than when swept from the plants with a net.

Sweep net best for most species.—The sweep net was by far the best equipment for collecting the most species of insects found in alfalfa, and for determining their comparative abundance during the season. It was very difficult to determine the effects of temperature and wind

TABLE V

NUMBER OF SPOTTED ALFALFA APHIDS TAKEN BY SWEEPS COMPARED TO NUMBERS CAUGHT BY FORK TOOL, PAYNE COUNTY, OKLAHOMA, MAY 29, 1955

AVERAGE NUMBER PER			EQUIVALENT			
Field Number	25 Sweeps	Fork Tool[a]	Sq. Inch Fork Tool	Sq. Inches[b] In 25 Sweeps	Temp.[c] °F.	Wind m. p. h.
1	967	226	53 2	18 2	74	12
2	222	72	16 9	13 1	68	12
3	591	110	25 9	22 8	70	5
4	52	3	0 7	74 3	64	8

[a]One for equals 4.25 square inches.
[b]Based on aphids per square inch in each field.
[c]On ground shaded by alfalfa plants.

conditions on the numbers collected because of natural weekly variations in the abundance of a species occurred at different times during a season. Table IV shows the records for 45 species very few of which, with the exceptions noted, were collected by other methods. This list is by no means complete, but includes all of those collected in sufficient numbers to be of interest. Certain of the Diptera and Hymenoptera were very abundant at times, but have not been identified so far. On the basis of 100 sweeps, which is the number taken for comparison in Table IV, each date represents approximately 12,500 tillers sampled. At no time during this period was any of these species, except aphids and thrips, sufficiently abundant to have occurred at the rate of one per tiller.

Comparison of sweeping and Henderson sampler.—A comparison of the sweep method with the fork sampling device of Henderson is shown in Table V. Four fields were sampled simultaneously with the net and the Henderson sampler. It was found that the latter was much faster. The spotted alfalfa aphid population varied from light to heavy. The numbers caught by the two methods varied in three fields to very nearly the same degree. However, the numbers taken in the field showing the lightest infestation were quite different as determined by the two methods. The fork device showed that the infestation in field number one was 73 times as heavy as in field number four, whereas the net showed it to be only 18 times as heavy. Much more of this field was covered by the 125 sweeps than by the ten spot samples taken by Henderson's sampler. Thus, the chances of missing a more heavily infested area by the latter device were much greater.

Comparison of sweeping with Berlese funnel samples.—The sweep net method of sampling was also compared with the one where surface soil, trash, and plant roots were processed with Berlese funnels. Comparisons were made (Table VI) of the numbers of spotted alfalfa aphids, thrips, *Frankliniella occidentalis* (Perg.), Collembola and mites, to show certain differences. More aphids and thrips were collected in the Berlese funnel samples than in the sweeps. However, one species of thrips, *Aelothrips bicolor* Hinds, was not taken in the soil and trash samples. The differences in numbers of mites and Collembola were striking, by far the most being collected by means of the Berlese funnel.

TABLE VI

COMPARISON OF SWEEP NET WITH BERLESE FUNNELS, NORTH ENTOMOLOGY FIELD, PAYNE COUNTY, OKLAHOMA, 1955

DATES AND METEOROLOGICAL CONDITIONS	INSECTS AND MITES	NUMBER PER 125 SWEEPS	NUMBER PER 17–20[a] SAMPLES
Mar. 29-31 Temp. 68°F. Wind-12 m.p.h.	aphids	2956	801
	thrips	8	59
	Collembola	0	51
	mites	2	67
Apr. 14 Temp. 66°F. Wind-5 m.p.h.	aphids	6624	1601
	thrips	93	171
	Collembola	0	47
	mites	1	97
Totals	aphids	9580	2402
	thrips	101	230
	Collembola	0	98
	mites	3	164

[a]Each sample consisted of one-half square foot.

SUMMARY AND CONCLUSIONS

One stroke with a sweep net on a handle 19 inches long sampled an average of 155 tillers in an alfalfa field where the stand was good. With the exception of the spotted alfalfa aphid and a species of thrips, from 44 to 77 percent of the insects resting on the plants were collected by this method. On the other hand not over 10 percent of these aphids resting on the plants were picked up by the net. Comparative figures also show that a maximum average of not over one

percent of the thrips were collected by the net. This was the best method of the five tested to collect the greatest number of species, particularly those which are winged and very active. The sweep net was an excellent tool for locating extremely light infestations. In somewhat heavier ones where the population distribution was very uneven this method proved to be very valuable.

Clipping plant tillers and immediately placing them in a jar for later examination was far more accurate than the net for determining the number of aphids and thrips present on the alfalfa plants. There was a large population of various species of insects and mites living on the surface of an alfalfa field which was best sampled with the pit trap or Berlese funnel methods. Especially abundant in this microhabitat were the ground beetles, Collembola and mites.

The Berlese funnel method was needed to determine larvae and other stages inhabiting the soil, and it also measured arthropod populations quantitatively which the pit trap did not. On the other hand, the pit trap sampled the soil surface of an alfalfa field for a much longer period than was possible by means of the Berlese funnel, and there is evidence that it will disclose the presence of certain very delicate forms which would not be found by the Berlese funnel.

The Henderson sampler was very well suited for taking samples of the spotted alfalfa aphid and usually caught them in the same proportionate numbers as the sweep net. It is concluded that all methods have their special uses and that a combination of several is needed to make a thorough and complete study of the arthropod ecology in an alfalfa field.

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NOTES ON AMERICAN MOSQUITO PUPAE II. THE AEDES (OCHLEROTATUS) PUNCTOR SUBGROUP, WITH KEY TO KNOWN NEARCTIC AEDES PUPAE

(DIPTERA, CULICIDAE)[1]

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In 1951, Knight published a study on the *punctor* subgroup of Edward's (1932) Group G of the subgenus *Ochlerotatus*. He presented evidence for the existence of five species in this complex; namely, *punctor* (Kirby), *hexodontus* Dyar, *aboriginis* Dyar, *punctodes* Dyar and *abserratus* (Felt and Young) (=*implacabilis* (Walker)). The positive identification of all of these members is possible only by larval characters, although adult morphology is helpful in many instances.

Since only about one-half of the pupae of Group G are known, and fewer in Groups F and H, it is impossible to characterize accurately these groups on the basis of pupae.

Subsequent work on all or parts of this difficult group has been completed by Becker (1954), Vockeroth (1954b), Frohne (1955a, b) and Carpenter and LaCasse (1955). Their findings have generally supported the observations of Knight.

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Through the kindness of Dr. Stone and Cmdr. Knight, many of the actual reared associations previously studied by Knight were made available for this investigation. An opportunity was afforded to evaluate his conclusions in the light of evidence contained in pupal characters.

It has been possible to prepare an identification key to the pupae of the species and varieties of the ***punctor*** complex. Pupae of ***hexodontus***, ***aboriginis*** and ***punctodes*** are described for the first time. *A. **punctor*** and ***abserratus*** are partially redescribed with the aid of additional specimens and abdominal sternal setae. Table I shows the range and mode of the number of branches occurring in the principal pupal setae of the five members of the complex. Dark-contrast, phase microscopy was used in the study.

KEY TO PUPAE OF PUNCTOR COMPLEX

1. 3–II single, seldom double; 4–III usually single to triple; 1–III generally unbranched; 6–VI single or double ..... **aboriginis**
— 3–II with 2 or more branches; 4–III usually with 4 or more branches; 1–III generally with 2 to 5 branches; 6–VI mostly triple or quadruple (double in *intrudens*) 2
2. A–VIII single or double; 1–VII mostly double or triple; 6–VII generally single or double 3
— A–VIII usually with more than 2 branches; 1–VII mostly with more than 3 branches; 6–VII generally triple or quadruple 4
3. B–IV double; MT–II and C–V usually double ..... **abserratus**
— B–IV single; MT–II and C–V usually single ..... **punctodes**
4. S–I about as long as T–I; B–IV single ..... **punctodes**
— S–I distinctly longer than T–I, mostly 1.5 to 2 times as long; B–IV generally double 5
5. A seta (8) present submedially on at least one side of abdominal sternite II; if not, then C–III double or triple and/or CT–8 pedunculate, at least basal one-twelfth unbranched ... **hexodontus**
   a. 3–II mostly single to triple; 4–III usually double to quintuple; MT–II generally double ..... "type **hexodontus**" var.
   aa. 3–II mostly 4– to 7–branched; 4–III usually with 6 or more branches; MT–II unbranched ..... "tundra" var.
— Sternite II without a submedian seta (8) on at least one side, or at most with trichopore only; but if present on both sides, then C–III with 4 or more branches and/or CT–8 branched from near base ..... **punctor**
   a. C–V, C–IV and MT–II usually double, sometimes triple or quadruple ..... "type **punctor**" var.
   aa. C–V, C–IV and MT–II mostly unbranched ..... "tundra" var.

DESCRIPTIONS AND DISCUSSIONS

Aedes (Ochlerotatus) punctor (Kirby)

The definition of the ***punctor*** pupa was first outlined by Clavero (1946). His key characters were A–VIII moderately branched; seta B bifurcate, large; seta C large and simple; and the respiratory trumpet with low-cut border (pinna). His Spanish material does not agree with Nearctic pupae in the latter two characters; C is mostly double (in "type ***punctor***" variety), and the pinna of the trumpet generally short, about one-fifth of the total length. Darsie (1951) has described this pupa more fully.

The original New York and Saskatchewan specimens and 36 additional pupae, primarily from Alaska, have been more completely studied. Besides extending the range in number of branches of many setae, the following changes in the author's original description have been noted.

CT–4 mostly 4– or 5–branched; *CT–8* not usually pedunculate (Fig. 7); respiratory trumpet with total length of 0.49 to 0.72 mm., averaging 5.88 times as long as pinna and 3.48 times as long as greatest diameter. On abdominal segment I, *M* usually with 5 to 7 branches; *S* averaging 1.84 times as long as *T*. On II, *1* and *3* generally triple or quadruple and *4* with 5 to 7 branches. On III, *2* mostly 4– to 6–branched; *4* with 5 to 7 branches. On IV, *B* single or double, *4* usually 4– or 5–branched. On V, *B* single or double, *2* generally quadruple or quintuple. *4–VI* mostly double. *B–VII* usually unbranched. *D* present on II in only 14 percent of the pupae studied, 2– or 3–branched; long on III–VII, primarily double on III–IV, simple on V–VII. *E* long, single, rarely double or triple on VI–VII. *6* short, pedunculate, mostly with 3 to 5 branches on III–VII. *7* short, pedunculate on III–V, generally 4– to 6–branched on III and V, triple or quadruple on IV. *8* present submedially at least on one side of sternum II as a minute to short, mostly unbranched seta in 39 percent of the specimens examined; in 42 percent, a vestige of this seta remains as a trichopore, in the center of which is a dark spot, sometimes slightly protruding; and in 18 percent, completely absent; simple, rarely double on III–VII. Paddle index 1.32 to 1.91, average 1.57.

Additional pupae studied were: ALASKA: 5 ♀♀ and 5 ♂♂ from Umiat (K. L. Knight and C. Schultz); 2 ♂♂ from Fairbanks-Circle, MP 32; 1 ♀ from N. of Fairbanks; 3 ♀♀ from Fairbanks; 3 ♀♀ and 1 ♂ from Glenn Highway (♂ from MP 174); 1 ♂ from Livengood Road; 1 ♀ and 1 ♂ from Richardson Highway, MP 172 and 258; 2 ♀♀ from Eklutna; 6 ♀♀ and 2 ♂♂ from Anchorage (collected by Alaska Mosq. Proj.). CANADA: 1 ♀ from Prince George, British Columbia (H. G. Dyar). USA: 2 ♀♀ from Dublin, New Hampshire (A. Busck).

Discussion: The existence of two forms within the ***punctor*** species has been suggested by Knight (1951). He named them "type ***punctor***" and "tundra" varieties. Larval as well as adult female characters were shown to possess differences. Frohne (1955a) substantiated Knight's larval characters for the "tundra" variety in a study of specimens from Naknek, Alaska, and added support with saddle spine morphology (1955b).

Knight and Carpenter and LaCasse (1955) both recognize that the "type ***punctor***" female is inseparable from ***aboriginis*** and ***abserratus***, and that the "tundra" female is not distinct from ***punctodes***, but they differ on the separation of females of the "tundra" varieties of ***punctor*** and ***hexodontus***. Knight states that they are generally distinct. The only character available for this separation is the few to many scattered pale scales anteriorly on the mid femur in "tundra" ***hexodontus***, and few to no pale scales in "tundra" ***punctor***. Carpenter and LaCasse evidently consider these two varieties indistinguishable. Beckel (1954) separates the females of ***punctor*** and ***hexodontus*** (sensus latus) on the basis of scalation of abdominal sternites, and bases of costa and radius. The male genitalia of ***punctor*** is apparently distinct from ***abserratus*** and ***punctodes***, but not from ***aboriginis*** and ***hexodontus***.

Knight believes that the larva of ***punctor*** (s. l.) is distinguishable from ***hexodontus*** (s. l.) and all other members of the complex. Additional larval characters are given by Vockeroth (1954b).

An exhaustive study of pupal characters has demonstrated the close relationship of ***punctor*** and ***hexodontus***. Nevertheless, by the use of the presence or absence of submedian seta 8 on abdominal sternite II, C–III and CT–8 complete separation has been achieved. In all of the ***punctor*** pupae studied, which have a well developed seta 8 on both sides of II, C–III is 4– to 10–branched and in most, CT–8 is not pedunculate (Figs. 7 and 8), while in all the ***hexodontus*** pupae in which 8 is not present on at least one side, C–III is double or triple and most of them have CT–8 unbranched in at least the basal one-twelfth.

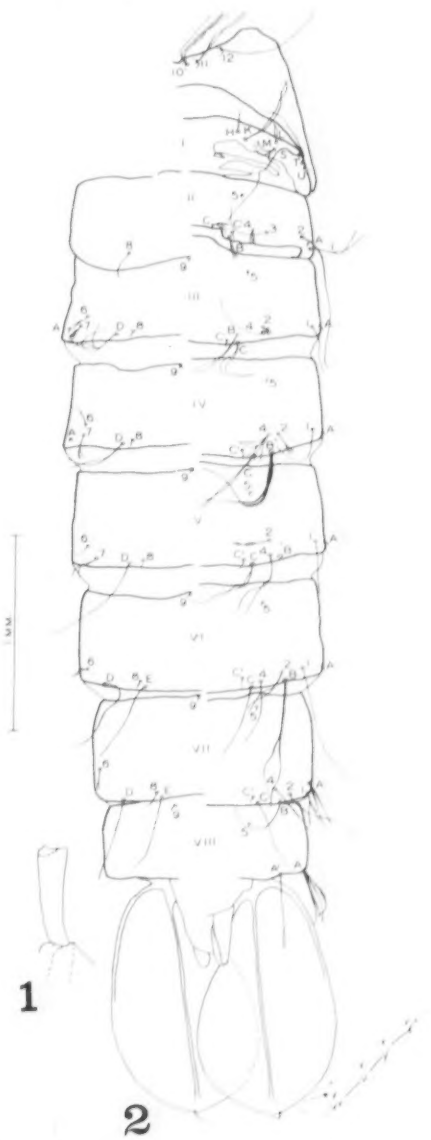
Specimens were available from Umiat, Alaska, to serve as examples of the "tundra" variety, and to compare with the remainder of the ***punctor*** pupae ("type ***punctor***" variety). On the basis of this relatively small sample, separation of the two varieties on at least a variety level was obtained. C–V in the "tundra" variety is 95 percent and C–IV, 80 percent single; in the "type ***punctor***" C–V is 82 percent and C–IV, 95 percent double to quadruple. Furthermore, CT–8 is pedunculate in 79 percent and MT–11 unbranched in 82 percent of the setae in "tundra" pupae, while CT–8 is branched from near base in 92 percent and MT–11 double to quadruple in 81 percent of "type ***punctor***" specimens.

Aedes (Ochlerotatus) hexodontus Dyar

Cephalothorax.—Ocular setae long, usually single or double; prothoracic seta *6* short, others long, mostly 2– to 4–branched; mesothoracic setae long, generally pedunculate with basal one-fourth to one-twelfth of *8* unbranched (Fig. 8); 8 usually double to quadruple, *9* single or double; metathoracic setae long, *11* and 12 pedunculate, *10* ordinarily with 4 or 5 branches, 11 simple, 12 bifurcate.

Respiratory trumpet (Fig. 5).—Usually increasing in diameter only slightly in apical half, surface reticulate with basal tracheoid patch; total length 0.56 to 0.76 mm., averaging 6.34 times as long as pinna, and 3.71 times as long as greatest diameter.

Abdomen (Fig. 6).—On I, reticulation on ter-



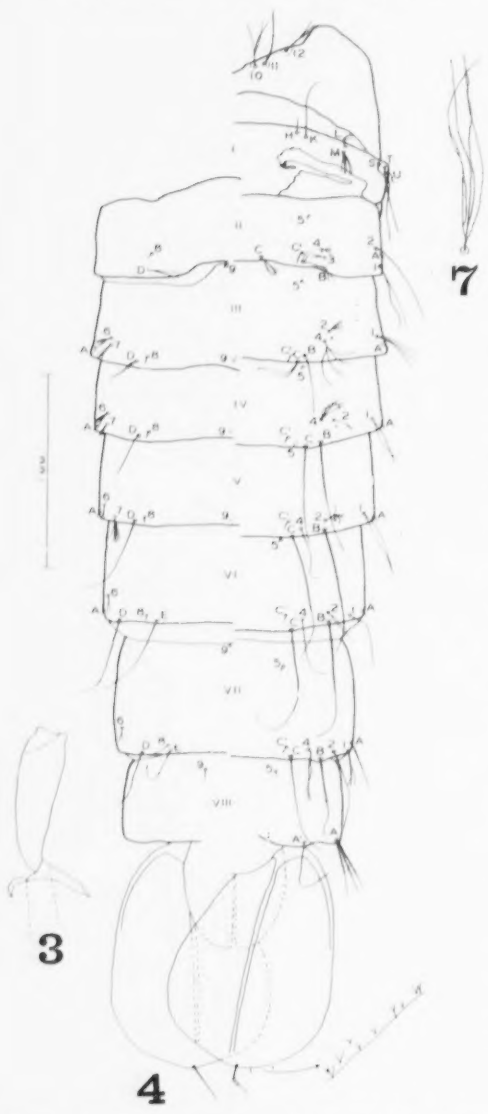
Figs. 1–2. Trumpet, and metathorax and abdomen of *Aedes aboriginis* Dyar (specimen W6 from Kwinitsa, B. C., Canada, ♀). Dorsal right and ventral left.

gum poorly developed or absent; *H* and *U* short, simple; *K* and *T* long, single or double; *S* very long, averaging 1.92 times as long as T, single; *L* and *M* medium, L ordinarily with 3 or 4 branches, M with 5 to 8 branches. *A* minute, simple, posterolateral on II–VI; rather stout, long, mostly double on VII; stout, long, sometimes dichotomously subdivided, usually with 3 to 5 branches on VIII. *A'* on VIII long, primarily single. *B* medium, pedunculate on II, long on III and VII, very long and stout on IV–VI; generally 2- or 3-branched on II, single or double on III–VI, mostly single on VII. *C* medium on II, long on III–VII; ordinarily double to quadruple on II–III, single or double on IV–VII. *D* present on II in only 13 percent of the pupae studied; medium on II, long on III–VII; simple to quadrifid on II, generally single or bifid on III–IV, simple on V–VII. *E* on VI–VII long, single. *1* long on II–VI, medium on VII; usually with 3 to 5 branches on II–III, single to triple on IV–V, single or double on VI, 4- to 6-branched on VII. *2* very long on II, short to medium, pedunculate on III–VI, long on VII; single, rarely double on II and VII, mostly 4- to 7-branched on III, 3- to 5-branched on V, 2- to 4-branched on IV and VI. *3* on II long, commonly bifid to quadrifid. *4* short on II, medium on III–IV, long V–VII; generally with 5 to 8 branches on II, 3- to 6-branched on III–IV, with 1 or 2 branches on V–VII. *5* and *9* minute, simple, medioanterior on terga II–VIII and sterna III–VIII, respectively. *6* short, pedunculate, usually triple to quintuple on II–VII. *7* short, pedunculate on III–V, mostly 4- to 7-branched on III and V, triple to quintuple on IV. *8* present on at least one side of sternum II in 80 percent of specimens examined, otherwise the seta represented by trichopore containing dark, sometimes protruding, center; short to medium, simple, occasionally 2- to 8-branched on II, unbranched on III–VII.

Paddle.—Ovoid, marginal and submarginal denticles border apical one-third, midrib not reaching apex; seta x rather stout, medium length, single, rarely double; index 1.23 to 1.74, average 1.46.

The description is based on the following material: ALASKA: 4 ♀♀ and 5 ♂♂ from Umiat (K. L. Knight—3 ♀♀ and 3 ♂♂; C. Schultz—1 ♀ and 1 ♂; L. H. Jachowski—1 ♂); 2 ♀♀ from Valdez-Fairbanks Highway, MP 23 and 200; 3 ♀♀ and 1 ♂ from Long L. on Richardson Highway, MP 131 and 148; 1 ♂ from Long L. on Richardson Highway; 1 ♂ from Glenn Highway, MP 35; 1 ♀ from Anchorage; (all from Alaska Mosq. Proj.). CANADA: 6 ♀♀ and 1 ♂ from Prince Rupert, British Columbia (H. G. Dyar, including type ♀ of *leuconotips* Dyar). USA: 1 ♀ from Truckee River, California (H. G. Dyar); 2 ♀♀ from Fallen Leaf Lake, Lake Tahoe, California (H. G. Dyar); 3 ♀♀ from Grand Lake, Colorado (H. G. Dyar).

Discussion: As in *punctor*, two varieties, "tundra" and "type *hexodontus*", were proposed by Knight (1951). He and Carpenter and LaCasse (1955) point out that the male genitalia of *hexodontus* (s.l.) are distinct from *abserratus* and *punctodes*, but not from *punctor* and *aboriginis*. For separation of the female of "tundra" variety



FIGS. 3–4. Trumpet, and metathorax and abdomen of *Aedes punctodes* Dyar (specimen B38C–3 from Anchorage, Alaska, ♂). Dorsal right and ventral left. FIG. 7. Cephalothoracic seta 8 of *Aedes punctor* (Kirby) (specimen 10G–30 from Ringwood, New York), 200x.

hexodontus from "tundra" ***punctor***, see discussion under ***punctor***. Knight and Carpenter and LaCasse also disagree on the differentiation of "type ***hexodontus***" variety and ***abserratus***; Carpenter and LaCasse distinguish them on the basis of the patch of white scales on the costa, present in the former and absent in the latter. Knight apparently considers these characters insufficient for their separation. They do concur that the ***punctodes*** and "tundra" ***hexodontus*** females are indistinguishable. Additional differences between females of ***punctor*** and ***hexodontus*** are indicated by Beckel (1954).

The larva has been defined by Knight as distinctive; in addition, he has differentiated the "tundra" from the "type ***hexodontus***" larvae. The latter separation has been supported partially by Frohne (1955a, 1955b). Vockeroth (1954b) added two more good larval characters to distinguish ***punctor*** and ***hexodontus***. He also included information on adults.

The ***hexodontus*** pupa is the least specific of the complex. There is more intergradation between the ranges of its setal branching and those of the other pupae of the complex than in any of the other members of this group. The characters used to distinguish the ***hexodontus*** pupa from ***punctor*** are enumerated in the discussion of the latter species and in the accompanying keys.

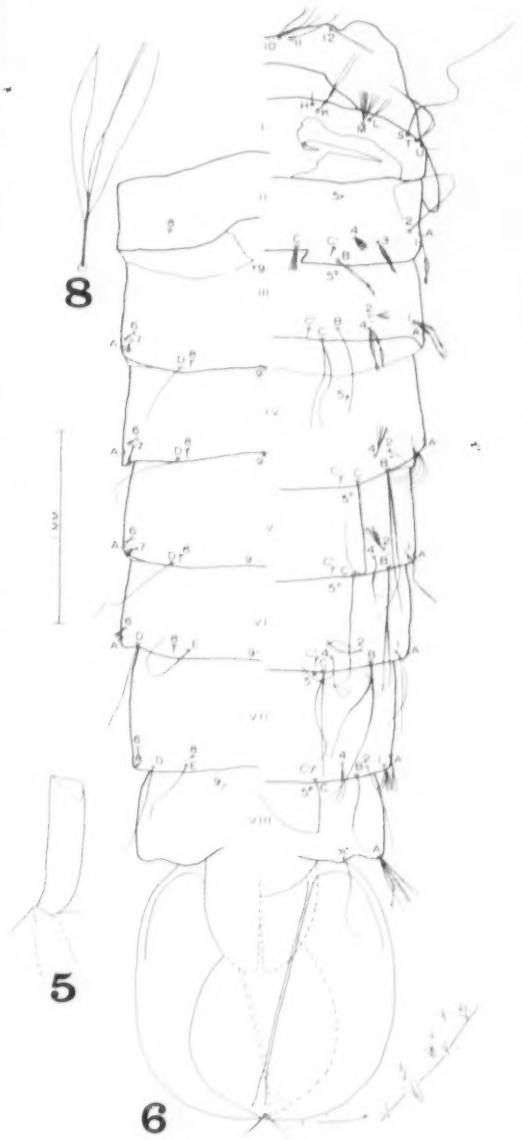
The pupae of ***hexodontus*** from Umiat, Alaska, representing the "tundra" variety are distinct from the "type ***hexodontus***" variety, which includes all other pupae of this species listed above. The degree of separation appears to justify at least Knight's variety status. The "tundra" pupae have MT–11 100 percent single, 3–II 80 percent with 4 to 7 branches, and 4–III with more than 5 branches; whereas "type ***hexodontus***" pupae have MT–11 60 percent double, 3–11 85 percent single to triple, and 4–III 84 percent with 5 or fewer branches. The "tundra" pupae have 8–II on at least one side in 89 percent, and "type ***hexodontus***", in but 76 percent.

Aedes (Ochlerotatus) aboriginis Dyar

Cephalothorax.—Ocular setae *1*, *2* and *3* usually single or double, long; prothoracic seta *6* short, others long, mostly bifid or trifid; mesothoracic setae *8* and *9* long, sometimes pedunculate, generally 1- to 3-branched; metathoracic setae long, smooth, *10* mostly with 4 or 5 branches, *11* and *12* double.

Respiratory trumpet (Fig. 1).—Constricted at base, increasing in diameter gradually toward apex; apex slightly emarginate; surface reticulate except for small semi-tracheoid patch near base; from 0.5 to 0.7 mm. long; total length averages 5.34 times length of pinna, and 3.28 times that of greatest diameter.

Abdomen (Fig. 2.)On segment I, reticulation on dorsum only rarely well developed, usually imperfect or absent; *H* and *U* short, simple; K and *T* long, generally double; *L* and *M* medium, L mostly double or triple, M triple or quadruple; *S* very long, single. *A* minute, simple, posterolateral on II–VI; well developed, non-plumose, usually double on VII; stout, long, slightly plumose, with 2 to 6 branches on VIII. *A'* on VIII long, single, seldom double. *B* medium on



FIGS. 5–6. Trumpet, and metathorax and abdomen of *Aedes hexodontus* Dyar (specimen 44-8 from Umiat, Alaska, ♂). Dorsal right and ventral left. FIG. 8. Cephalothoracic seta 8 of *A. hexodontus* (spec. 44-8), 200x.

II, long on III and VII, very long, stout on IV–VI; commonly trifid on II, bifid on III–VI, simple on VII. *C* of medium development on II, long on III–VII; usually 5- to 7-branched on II, triple on III, double on IV–VI, single on VII. *C'* short, simple, medioposterior on II–VII. *D* absent on II, long on III–VII; mostly double on III and simple on IV–VII. *E* long, simple on VI–VII. *I* long on II–VI, medium on VII; ordinarily bifid on II, single on III–VI, 2- to 4-branched on VII. *2* very long on II, short on III, medium on IV–V, long on VI–VII, pedunculate when branched; single on II and VII, generally quadruple on III, double or triple on IV–VI. *3* long, single, rarely double on II. *4* short on II, medium on IV, long on III and V–VII; usually 4- to 6-branched on II and IV, single or double on III and V–VII. *5* and *9* minute, simple, medioanterior on terga II–VIII and sterna III–VIII, respectively. *6* short to medium, pedunculate on III–VII; commonly single to triple on III–VII. *7* short, ordinarily quadrifid on III and V, bifid or trifid on IV. *8* present on II in 27 percent of specimens examined, short, simple on II–VII.

Paddle.—Ovoid, marginal and submarginal denticles on apical one-third, midrib not reaching apex, seta *x* single, rarely double; index 1.38–2.02, average 1.65.

The description is based on 10 ♀ ♀ and 4 ♂ ♂ from Longmire Springs, Mt. Ranier National Park, Washington (H. G. Dyar); 6 ♀ ♀ and 2 ♂ ♂ from Prince Rupert, British Columbia, Canada (H. G. Dyar), 2 ♀ ♀ from Kwinitsa, British Columbia, Canada (H. G. Dyar).

Discussion.—Knight (1951) and Carpenter and LaCasse (1955) report that females of *aboriginis* are indistinguishable from *abserratus* and "type" variety of *punctor*, and males of *aboriginis*, from *punctor* and *hexodontus*. Knight indicates that the larvae are quite distinct; also, Frohne (1955b) has defined *aboriginis* larvae on the basis of the saddle spines. In the pupal stage, this species is the most distinctive of the complex. In addition to the key characters, *aboriginis* may be separated from *abserratus* and *punctodes* by A–VII, double or triple in the former and simple in the latter two; also C–II is mostly 5- to 8-branched in *aboriginis* and 2- to 4-branched in *abserratus*.

Aedes (Ochlerotatus) punctodes Dyar

Cephalothorax.—Ocular setae long, slender, single to triple; prothoracic seta *6* medium, others long, *4* mostly 3- or 4-branched, others bifid or trifid; mesothoracic setae long, pedunculate, *8* usually with 3 or 4 branches, *9* simple; metathoracic setae long, *11* sometimes sparsely plumose, generally single, *10* and *12* double or triple.

Respiratory trumpet (Fig. 3).—Gradually widening toward apex, slightly emarginate at apex, surface reticulate with thin tracheoid patch near base; total length from 0.47 to 0.73 mm., averaging 6.02 times as long as pinna, 3.68 times as long as greatest diameter.

Abdomen (Fig. 4).—On I, reticulation on dorsum poorly developed or absent; *H* and *U* short, simple; *K* and *T* long, usually double; *S* long, averaging 0.85 times as long as T, single; *L* and M medium, L with 3 to 5 and M ordinarily with 4 to 6 branches. *A* minute, simple, posterolateral on II–VI; rather stout, long, single, seldom double or triple on VII; stout, long, somewhat plumose, usually 2- or 3-branched on VIII. *A'* on VIII long, simple. *B* medium on II, long on III and VII, very long on IV–VI; generally double or triple on II, single or double on III, almost always single on IV–VII. *C* medium on II, long on III–VII; mostly with 2 to 5 branches on II, double on III, single, seldom double on IV–VII. *D* present on II in only 6 percent of the pupae studied, 2- or 3-branched; long on III–VII; usually bifid on III, single or bifid on IV, single on V–VII. *E* long, simple on VI–VII. *I* long on II–VI, medium on VII; mostly double to quadruple on II and IV, with 3 or 4 branches on III and VII, single or double on V, single on VI. *2* very long on II, short to medium on III–VI, long on VII; single, sometimes double on II and VII, generally with 4 to 6 branches on III and V, triple or quadruple on IV, double or triple on VI. *3* on II medium, commonly 2- or 3-branched. *4* short on II, medium on III–IV, long on V–VII; ordinarily with 4 to 6 branches on II–IV, simple on V–VII. *5* and *9* minute, simple, medioanterior on terga II–VIII and sterna III–VIII, respectively. *6* short to medium, usually double to quadruple on III–VII. *7* short to medium, mostly 3- to 5-branched on III–V. *8* present, small, simple on II in 87 percent of specimens examined; small, simple, rarely double on III–VII.

Paddle.—Ovoid, submarginal denticles on apical one-third, midrib not reaching apex; seta *x* medium, simple; index 1.33–1.85, average 1.56.

The description is based on 10 ♀ ♀ and 4 ♂ ♂ from Anchorage, Alaska (Alaska Mosq. Proj.—♂ ♂ collected by B. V. Travis—2, Gjullin—1, A. Stone—1); 1 ♀ from Eklutna, Alaska (Alaska Mosq. Proj.).

Discussion.—Knight (1951) and Carpenter and LaCasse (1955) hold that the male of *punctodes* is inseparable from *abserratus*, and the female is not distinct from the "tundra" varieties of *punctor* and *hexodontus*. The larva has been found by Knight to be distinct from all other members of the complex. In a study of the larval saddle spines, Frohne (1955b) was able to distinguish *punctodes* from the "tundra" forms of *punctor* and *hexodontus*.

The pupa of *punctodes* is distinct from the other pupae of the complex. Its most distinct features are the single B–IV and the S about as long as T on abdominal segment I. In the keys, *punctodes* comes out at two places. About 55 percent of

TABLE I

RANGE OF BRANCHING IN SETAE OF *Aedes punctor* COMPLEX PUPAE (MODES IN PARENTHESES)

SETA	*aboriginis*	*abserratus*	*punctodes*	*hexodontus*	*punctor*
Cephalothorax					
1	1-3 (2)	1-3 (2)	1-3 (2)	1-3 (2)	1-3 (2)
2	1-3 (2)	2 (2)	1-3 (2)	1-3 (2)	1-4 (2)
3	1-2 (1)	1-2 (1)	1-3 (2)	1-2 (1)	1-3 (1)
4	2-6 (3)	2-4 (3)	2-5 (3)	2-6 (3)	2-7 (4)
5	2-5 (2)	2-4 (3)	2-4 (2)	2-4 (3)	2-7 (3)
6	1-5 (3)	2-4 (?)	2-4 (2)	1-6 (3)	1-6 (4)
7	2-3 (2)	2-4 (3)	1-5 (2)	1-5 (3)	1-6 (3)
8	1-5 (3)	2-5 (3)	2-6 (4)	1-7 (2)	3-8 (4)
9	1-2 (2)	1-3 (2)	1-2 (1)	1-3 (2)	1-3 (2)
Metathorax					
10	3-8 (5)	2-6 (3, 4)	2-7 (?)	2-7 (4)	3-10 (5)
11	1-3 (2)	1-4 (2)	1-2 (1)	1-2 (1)	1-4 (2)
12	1-4 (2)	1-3 (2)	2-3 (2)	1-5 (2)	1-4 (2)
Abdomen I					
H	1 (1)	1 (1)	1 (1)	1-3 (1)	1-4 (1)
K	2-3 (2)	1-3 (2)	1-3 (2)	1-3 (2)	1-4 (2)
L	2-6 (3)	2-5 (3)	3-5 (3)	2-6 (4)	2-6 (3)
M	3-7 (4)	4-7 (6)	4-8 (?)	3-10 (6, 7)	4-9 (6)
S	1-2 (1)	1-2 (1)	1-2 (1)	1-2 (1)	1-2 (1)
T	1-3 (2)	1-2 (2)	1-3 (2)	1-4 (2)	1-4 (2)
U	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)
II					
B	2-5 (3)	2-4 (2)	1-3 (2)	1-5 (2)	1-5 (2)
C	3-10 (6)	2-4 (2)	2-8 (?)	1-7 (3)	2-15 (4)
D			2-3 (?)	1-4 (?)	2-3 (?)
1	2-4 (2)	1-4 (2)	2-6 (2)	1-7 (3)	2-6 (3, 4)
2	1-2 (1)	1-2 (1)	1-2 (1)	1-2 (1)	1-3 (1)
3	1-2 (1)	1-4 (3)	1-3 (2)	1-7 (2, 3)	1-6 (4)
4	3-8 (5)	3-6 (6)	2-9 (6)	3-11 (7)	5-9 (6)
8	1 (1)	1 (1)	1-2 (1)	1-8 (1)	1 (1)
III					
B	1-3 (2)	1-3 (2)	1-4 (1)	1-2 (1)	1-4 (2)
C	1-4 (3)	1-5 (2)	1-7 (2)	1-7 (3)	2-10 (4)
D	1-4 (2)	1-3 (2)	1-3 (2)	1-4 (3)	1-4 (2)
1	1-3 (1)	1-4 (3)	1-4 (3)	1-6 (2)	1-5 (3)
2	1-6 (4)	2-8 (?)	2-8 (5)	4-10 (5)	3-9 (6)
4	1-6 (2)	2-6 (3)	2-6 (4)	2-9 (6)	2-9 (6)
6	1-5 (2)	2-4 (3)	2-8 (3)	2-6 (3, 4)	2-7 (4)
7	2-6 (4)	2-6 (4)	3-7 (4)	2-8 (5)	1-10 (5, 6)
IV					
B	2 (2)	1-2 (2)	1-2 (1)	1-3 (2)	1-3 (2)
C	1-3 (2)	1-3 (2)	1-2 (1)	1-4 (1)	1-4 (2)
D	1-3 (1)	1-3 (2)	1-2 (1)	1-4 (2)	1-4 (2)
1	1-2 (1)	1-2 (1)	1-4 (2)	1-5 (2)	1-5 (2)
2	1-3 (2)	1-4 (2)	2-5 (3)	1-6 (2)	1-6 (3)
4	2-7 (4)	3-7 (4)	3-7 (5)	3-7 (4)	3-7 (4)
6	1-4 (1)	1-4 (2)	1-4 (3)	2-4 (3)	1-6 (3)
7	1-3 (2)	2-4 (3)	2-5 (3)	1-6 (3)	2-5 (3)

TABLE I—(Continued)

SETA	*aboriginis*	*abserratus*	*punctodes*	*hexodontus*	*punctor*
			V		
B	2-3 (2)	1-3 (2)	1-2 (1)	1-3 (2)	1-3 (1)
C	1-3 (2)	1-3 (2)	1-2 (1)	1-3 (1)	1-4 (2)
D	1 (1)	1 (1)	1-2 (1)	1 (1)	1-2 (1)
1	1-3 (1)	1-2 (1)	1-3 (1)	1-4 (1)	1-4 (1)
2	2-5 (3)	2-5 (4)	3-7 (5)	2-7 (5)	3-9 (5)
4	1-3 (2)	1-3 (2)	1-3 (1)	1-2 (2)	1-6 (2)
6	1-3 (1)	1-3 (2)	2-6 (3)	2-5 (3)	2-7 (3)
7	2-5 (4)	3-6 (4)	3-6 (5)	1-8 (4)	2-10 (5)
			VI		
B	1-3 (2)	1-2 (2)	1-2 (1)	1-3 (2)	1-3 (1)
C	1-3 (2)	1-3 (2)	1-2 (1)	1-3 (1, 2)	1-4 (2)
D	1-2 (1)	1-2 (1)	1 (1)	1 (1)	1-2 (1)
E	1 (1)	1 (1)	1 (1)	1 (1)	1-3 (1)
1	1-6 (1)	1-2 (1)	1-2 (1)	1-2 (1)	1-4 (1)
2	1-3 (2)	1-4 (3)	1-5 (2)	1-5 (2)	2-4 (3)
4	1-2 (1)	1-3 (2)	1-2 (1)	1-3 (2)	1-3 (2)
6	1-3 (2)	1-3 (2)	2-4 (3)	1-5 (3)	2-5 (3)
			VII		
A	1-4 (2)	1 (1)	1-3 (1)	1-3 (2)	1-3 (1)
B	1-3 (1)	1-3 (1)	1 (1)	1-2 (1)	1-2 (1)
C	1-3 (1)	1-2 (1)	1-2 (1)	1-2 (1)	1-3 (2)
D	1-5 (1)	1-2 (1)	1-2 (1)	1 (1)	1-2 (1)
E	1 (1)	1 (1)	1 (1)	1 (1)	1-2 (1)
1	2-6 (3)	2-6 (3)	2-6 (3)	3-9 (4, 5)	2-9 (5)
2	1-2 (1)	1-2 (1)	1-2 (1)	1-3 (1)	1-3 (1)
4	1-3 (2)	1-3 (2)	1-2 (1)	1-3 (2)	1-4 (2)
6	1-4 (2)	1-3 (2)	1-4 (3)	1-6 (3)	2-6 (4)
			VIII		
A	2-6 (3)	1-3 (1)	1-7 (2)	2-7 (4)	1-7 (3)
A'	1-2 (1)	1-2 (1)	1 (1)	1-3 (1)	1-2 (1)
			Paddle		
x	1-2 (1)	1 (1)	1 (1)	1-2 (1)	1 (1)

the specimens studied will key to couplet 3 and the remainder, to couplet 4.

There is no question about the closer resemblance of the *punctodes* pupa to the "tundra" varieties than to the "type" varieties of *punctor* and *hexodontus*. In the characters used to distinguish the "tundra" from "type" forms in the discussions of these two species above, *punctodes* invariably agrees with the "tundra" form.

Aedes (Ochlerotatus) abserratus
(Felt and Young)

The pupa of this species was described under *Aedes* (*Ochlerotatus*) *implacabilis* (Walker) by Darsie (1951); however, the holotype was found to be a female of *punctor* (Kirby) by Vockeroth (1954a). The correct name then became *abserratus* (Felt and Young).

The original material and five additional pupae were studied. The following changes in the original description have been made.

Respiratory trumpet with total length of 0.56 to 0.66 mm., averaging 5.64 times as long as pinna, and 3.38 times as long as greatest diameter. *MT–10* and *L–1* mostly triple or quadruple; *C–II* chiefly double or triple; *1–II* usually double; *2–III* with 3 to 6 branches; *C–VI* simple to trifid; *2–VI* ordinarily double or triple; *1–VII* medium, mostly bifid or trifid; *D* absent on II, long on III–VII, double or triple on III–IV, simple on V–VII. *6* short, pedunculate, chiefly 2- or 3-branched on III–VII. *8* present on II in only 14 percent of the pupae studied.

Additional pupae examined were 1 ♀ and 1 ♂ from Ringwood, Dryden, New York (RFD) and 3 ♀♀ from Dublin, New Hampshire (A. Busck).

Discussion.—According to Knight (1951) and Carpenter and LaCasse (1955), the male genitalia of ***abserratus*** are indistinguishable from those of ***punctodes***. From information given by Knight, the female of ***abserratus*** is inseparable from ***aboriginis*** and "type ***punctor***" variety. It is distinct from both varieties of ***hexodontus***; see discussion under ***hexodontus***. Knight comments about the female of ***abserratus***, "when one is familiar with the fine reddish-brown median scutal scaling typical of ***implacabilis*** [= ***abserratus***], the female is apparently always recognizable." This is partially confirmed by Vockeroth (1954b) in discussing separation of this female and ***hexodontus***.

There appears to be little difficulty in separating ***abserratus*** from the other members of the ***punctor*** complex in the pupal stage. The 2- or 3-branched I–VII and the single or double A–VIII are the most distinctive features.

IDENTIFICATION OF KNOWN NEARCTIC AEDES PUPAE

In 1951, the author published a key for the identification of *Aedes* pupae. Although it included primarily the species from the Northeastern United States, all Nearctic pupae known at that time were incorporated. (Exceptions; ***dorsalis*** (Meig.), ***dupreei*** (Coq.), ***mitchellae*** (Dyar) and ***tormentor*** D. & K. listed by Mitchell (1907) in her identification key—she did not use the modern concept of chaetotaxy, nor were specimens of these species available for study). Since that time, seven more *Aedes* pupae have been described: ***diantaeus*** H., D. & K. and ***decticus*** H., D. & K., by Smith (1952); ***riparius*** D. & K. and ***pionips*** Dyar, by Darsie (1955); and ***aboriginis***, ***hexodontus*** and ***punctodes*** herein. Smith (1952) described ***decticus*** as ***pseudodiantaeus***, which subsequently was synonymized by Carpenter and LaCasse (1955). These species have been incorporated in the key, bringing the total to 27.

Certain aspects of the original key were troublesome. Couplet 13 was especially weak and it has been eliminated as such in the revision. The pupa of ***canadensis*** was surprisingly difficult to distinguish from some of the black-legged *Aedes*, especially in view of the distinctiveness of its other stages.

Smith (1952) placed ***decticus*** correctly in the pupal key; however ***diantaeus*** does not belong in couplet 6. Pupae examined by the author have had C–III–VII long, considerably longer than one-half the length of the following tergum; and MT–10 is 4- to 6-branched. In the revised key, ***diantaeus*** runs through couplets 9 and 15 to 16, where the 2- to 6-branched H–I and subequal A–VII and I–VII distinguish it from the subsequent species.

KEY TO KNOWN NEARCTIC AEDES PUPAE

1. A–II–VI well developed, rather stout; CT–6 long, stout; 4–IV usually double, seldom triple ..... **aegypti** (Linné)
— A–II–VI minute, not well developed; CT–6 short to medium; 4–IV usually with 3 or more branches 2
2. B–VII small, usually 3- to 5-branched; respiratory trumpet generally 4 or fewer times as long as its pinna 3
— B–VII medium to long, usually single or double; trumpet generally more than 4 times as long as its pinna 4
3. Paddle as wide as, or wider than long, apex emarginate, margin smooth; C–II with 4 to 8 branches ..... **atropalpus** Coq.
— Paddle longer than wide, apex rounded, margin partially denticulate; C–II with 13 to 30 branches ..... **trivittatus** Coq.
4. C–II with 20 to 34 branches; MT–10 usually 14-branched; respiratory trumpet about 3 times as long as its pinna ..... **taeniorhynchus** Wied.
— C–II usually 2- to 11-branched; MT–10 generally 2- to 12-branched; trumpet mostly 5 or more times as long as its pinna 5
5. 4–III and C–III single, rarely double; C–IV–VI simple; S–I and 2–II reduced in size, medium to long, branches very slender ..... **trichurus** Dyar
— 4–III usually 3- to 5-branched; C–III generally more than double; C–IV–VI usually 2-branched or more 6
6. C–III–VII reduced in length, not more than half the length of following tergum; 4–V usually single 7
— C–III–VII usually long, mostly much more than half the length of following tergum; 4–V usually double or triple 8
7. Reticulation between float hairs on abdominal segment I absent; 2–II about as long as tergum II; A–VIII with four or more branches ..... **triseriatus** (Say)
— Reticulation between float hairs on I present; 2–II about one-half as long as tergum II; A–VIII single to triple ..... **decticus** Howard, Dyar & Knab
8. B–II–VI almost invariably single (B–IV occasionally double or triple); A–VIII single, rarely double; A–VII simple ..... **cinereus** Meig.
— B–II–VI never all single; A–VIII usually with 2 or more branches; A–VII generally double to quadruple 9
9. C–IV and C–V usually not less than triple; 1–VI generally double; 1–IV usually not less than triple; 3–II generally 4- or 5-branched 10
— C–IV and C–V usually single or double; 1–VI single, sometimes double; 1–IV usually single or double; 3–II generally double or triple 15
10. Lines of reticulation between float hairs on tergum I heavy; C–II usually 5- or 6-branched 11
— Lines of reticulation between float hairs on tergum I thin or absent; C–II usually 11-branched 14
11. B–IV usually quadrifid; MT–10 generally with 6 or more branches; C–VI mostly 4-branched ..... **aurifer** Coq.
— B–IV double, rarely triple; MT–10 generally with 5 or fewer branches; C–VI mostly double 12
12. MT–11 single, rarely double; L–I generally double; 1–II usually triple; respiratory trumpet about 7 to 8 times as long as its pinna ..... **fitchii** F. & Y.
— MT–11 double, rarely single; L–I with 3 or more branches; 1–II usually double; trumpet about 6 times as long as its pinna 13

13. C–VII seldom with less than 2 branches; 4–VII usually double; B–II generally triple ..... **excrucians** (Walk.)
— C–VII single, seldom double; 4–VII usually triple, B–II generally double ..... **riparius** D. & K.
14. K–I simple; 4–VII usually 4–branched; 1–VII generally triple; paddle margin mostly smooth; B and C on IV–VI usually not as long as following tergum ..... **vexans** Meig.
— K–I double or triple; 4–VII usually double; 1–VII generally 8–branched; paddle margin denticulate; B and C on IV–VI longer than following tergum ..... **cantator** Coq.
15. S on abdominal segment I and 2 on II mostly double to quadruple; T usually longer than S on I ..... **communis** DeG.
— S–I and 2–II mostly single; T usually longer than S on I 16
16. H–I with 2 or more branches; setae A and 1 on abdominal segment VII subequal in length ..... **diantaeus** H. D. & K.
— H–I almost always simple; seta A distinctly longer, generally one and one-half times or more longer than 1 on VII 17
17. MT–12 usually 4– to 6–branched; CT–3 mostly double; paddle index (length divided by width) generally 1.35 or smaller 18
— MT–12 usually single or double; CT–3 mostly simple; paddle index generally 1.4 or larger 19
18. A–VII usually with 3 to 5 branches; 1–III generally single ..... **stricticus** (Meig.)
— A–VII usually single or double; 1–III generally double to quadruple ..... **pionips** Dyar
19. A–VII with 4 to 8 branches; K–I mostly single ..... **sollicitans** Walk.
— A–VII single to triple; K–I mostly double or triple 20
20. A–VIII usually with 6 or more branches; reticulation on abdominal tergum I present, lines heavy ..... **stimulans** (Walk.)
— A–VIII usually with 5 or fewer branches; reticulation on I present or absent, if present, lines light 21
21. 3–II single, seldom double; 4–III usually single to triple; 1–III generally simple; 6–VI single or double ..... **aboriginis** Dyar
— 3–II with 2 or more branches; 4–III usually with 4 or more branches; 1–III generally with 2 to 5 branches; 6–VI mostly triple or quadruple (double in *intrudens*) 22
22. A–VIII single or double; 1–VII mostly double or triple; 6–VII generally single or double 23
— A–VIII usually with more than 2 branches; 1–VII mostly with more than 3 branches; 6–VII generally triple or quadruple 24
23. B–IV double; MT–11 and C–V usually double ..... **abserratus** (Felt and Young)
— B–IV single; MT–11 and C–V usually single ..... **punctodes** Dyar (p. p.)
24. S–I about as long as T–I; B–IV single 25
— S–I distinctly longer than T–I, mostly 1.5 to 2 times as long; B–IV generally double 26
25. B–IV and A–VII almost always double; 4–VII usually double or triple ..... **intrudens** Dyar
— B–IV and A–VII almost always single; 4–VII generally single ..... **punctodes** Dyar (p. p.)
26. C–II mostly with 7 or more branches; 6–VI usually single or double; 1–II generally double ..... **canadensis** (Theo.)
— C–II mostly with 6 or fewer branches; 6–VI and 1–II generally triple or quadruple 27
27. A seta (8) present submedially on at least one side of abdominal sternite II, if not, then C–III double or triple and/or CT–8 pedunculate, at least basal one-twelfth unbranched ..... **hexodontus** Dyar
— Sternite II without a submedian seta (8) on at least one side, at most with trichopore only present, if seta on both sides, then C–III with 4 or more branches and/or CT–8 branched from near base ..... **punctor** (Kirby)

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NOTICE TO AUTHORS

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BIOLOGY OF DIPOGON SAYI BANKS (HYMENOPTERA, POMPILIDAE) IN TRAP-NESTS IN WISCONSIN

J. T. MEDLER AND T. W. KOERBER[1]

Many of the trap-nests placed in the field for research primarily on Megachilidae were found to be utilized by the spider wasp, *Dipogon sayi* Banks.[2] Apparently little has been added to the literature[3] on this species since the Peckhams (1898) reported on the biology of *sayi* (= *Pompilus calipterus* Say) as follows: "It was on the fifth of August that we saw this good sized, dark gray wasp, walking up and down a fence post. A little scrutiny showed that she was carrying pellets of earth from the ground into a hole four feet above, in the wood, and very odd she looked, making this journey again and again on foot instead of using her wings. Upon cutting away the post we found that she was just partitioning off the second apartment in what was doubtless intended to be a long series. Both cells contained female spiders of the species *Xysticus ferox*, that in the inner one being twice as large as the other, and each spider had an egg on the side of the abdomen. The large one was dead, but the other responded feebly to stimulation and lived in this state for four days. One of the eggs, the one that had just been laid when we opened the nest, hatched at the end of sixty hours, but the larva did not live."

The trap-nest technique has enabled us to accumulate considerable data which otherwise would be available rarely through the chance observations of field naturalists. Although incidental to the major objectives of the trap-nest research, it seems appropriate at this time to report upon the nesting habits of *D. sayi* in Wisconsin.

METHOD

The research was conducted with the types of trap-nests described by Medler and Fye (1956). The domicile-types were used mostly at the Ashland Branch Experiment Station in 1952, 1953 and 1954. However, during 1955 and 1956, sumac bundles were used entirely, and other localities were added in addition to those first used only in northern Wisconsin. The sumac trap-nests were made by bundling together several 8-inch lengths of sumac stems, the soft pith of which had been cleared with a $\frac{1}{4}$-inch (6.5 mm.) or $\frac{5}{16}$-inch (7.8 mm.) drill to a depth of 6 inches. Also, this species readily utilized bamboo stems when bundles of such stems were tested in 1952, providing the inside diameter of a bamboo section was approximately 6 mm.

The trap-nests were placed at the various field stations in early spring and inspected several times during the summer. The stems with finished nests were identified by orifice plugs, and unfinished nests were ascertained by probing with a straw. These stems were removed from bundles, identified by a code number, and brought back to the laboratory to be opened and studied. A standard data sheet was used for each nest. A sketch of the nest and the records relating to it were entered on the data sheet. In many instances the content of a cell was transferred to a small glass vial for rearing, or for time-temperature studies. In some instances the large collections necessitated capping a stem with a glass vial and merely holding it for the emergence of adult wasps. Such stems were opened later and the construction of the nest studied, but exact association of adults with cells could not be made.

RESULTS

During the five-year period, 1952–1956, a total of 221 nests were examined. A finished nest consisted of an array of cells separated by partitions and closed by plugs. A wide variety of organic and inorganic building material was used in constructing the partitions and plugs, including sawdust, wood chips, chewed leaf, lichen, moss, pieces of grass or leaves, clay, soil and sand grains. The partitions, which ranged between 0.5–5 mm. thick, were usually made with clay, soil, and sand grains and macerated pieces of organic matter. The exterior side of a partition (cell bottom) was concave and cemented smoothly so that often the face had a glassy appearance. The plugs either were made with sawdust, small chips, or miscellaneous plant material, which was loosely packed except for the smoothly cemented exterior face, or were made almost entirely with compacted clay, soil and sand grains. A distinctly layered plug was sometimes found having the exterior face of each layer cemented before the next layer was added. However, plugs were found with loosely packed plant material which lacked the cemented face. A plug was used to close the last cell, and another plug also was made in order to form an empty

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[2]We wish to express our appreciation to R. R. Dreisbach and Karl V. Krombein, for examining many of our reared wasps; only this species of *Dipogon* was found among them.

[3]Townes, (1957), has published on the taxonomy and distribution of this species, including biological notes.

vestibule of varying length. The vestibular plug was frequently, but not always, at the hole orifice. Vestibules were lacking in some nests which had the appearance of a finished nest. It is possible that the wasp did not complete her final plugging activity because of our removal of the nest, but in an analysis of data all nests without the vestibule and orifice plug were considered as unfinished nests. We never observed a nest having a plug placed at the inner end of the boring prior to provisioning of the first cell.

The nests obtained in our collections had a rather wide diversity of construction. In Table I, detailed records of selected nests are given which show the variation we found in the number of cells, length of cells, sequence of sexes, mortality, and parasitism. The number of cells in 41 finished nests of the first generation was 3.2 ±1.47, range 1–6; in 107 finished nests of the second (overwintering) generation 4.7±2.52, range 1–13. The number of cells in all finished nests was 4.3±2.38. The cells in 73 unfinished nests were 4.1±2.44, range 1–12.

Eighty-five percent of the nests in the sumac bundles were constructed in holes having an $^1/_4$ inch diameter, indicating a much greater choice of that size over the $^5/_{16}$ inch holes which were available in the same bundle. Although the preferred size of hole for nesting is probably $^1/_4$ inch, the species will utilize smaller holes, since two nests found in nature were in dead sumac stems with inside diameters of 5 and 6 mm.

The nests of *D. sayi* were provisioned exclusively with spiders. Unconsumed spiders or spider remains sampled from nests were identified as follows:[4]

AMAUROBIIDAE
Amaurobius sp.

GNAPHOSIDAE
Nodocion melanie Levi
Poecilochroa capulata (Walckenaer)
P. montana [illegible]rton

SALTICIDAE
Phidippus whitmani Peckham

THOMISIDAE
Coriarachne versicolor Keyserling
Misumena vatia Clerck
Misumenoides formosipes (Walckenaer)
Tmarus angulatus (Walckenaer)
Xysticus bicuspis Keyserling
X. elegans Keyserling
X. ferox (Hentz)
X. pellax O. P. Cambridge
X. triguttatus Keyserling

All of the spiders sampled were adult or juvenile females, the adults being twice as numerous as juveniles. The majority were species in the genus *Xysticus*. These thomisid spiders are fairly abundant on the ground or on trees and fallen logs in wooded areas. They do not spin webs, but pounce upon their prey from ambush. In nature, the female wasp can be seen running swiftly over the ground and vegetation or making short, darting flights close to the ground. Presumably she is searching for spiders at these times.

Invariably each cell was provisioned with a single intact spider. A creamy white egg about 1 mm. long was glued to the anterior portion of the side of the spider's abdomen. The newly hatched larva began feeding at the site where the egg was attached. Under the microscope it was possible to observe internal organs and watch food moving through the digestive tract of the almost transparent larva.

As the larva developed it became a cloudy gray color, and as it neared maturity numerous white granular bodies were seen under the still transparent cuticle. The larva remained attached by its posterior end to the abdomen of the spider until about half grown. During this time it sucked juices through a small hole in the integument of the spider at the place where feeding was first started. Upon reaching the proper stage of growth the larva began to chew rather than suck at its food. The abdomen of the spider was consumed first, followed by the cephalothorax. The legs were eaten last, but sometimes were left if the spider was large. The individual spiders differed in size, and in consequence of the different amounts of food available the mature larva varied from 4 to 10 mm. in length. Before cocooning was started the larva had a distinctly segmented form. Figures 1 to 6 show the development from egg to mature larva.

A thin white or creamy-colored oval cocoon, rounded at both ends and somewhat tapered posteriorly, was spun with silk by the mature larva. Upon completion of the cocoon the larva passed a meconium which soaked through the narrow end of the cocoon causing it to adhere firmly to the plug of the cell, or occasionally the wall of the cell.

At the prepupal stage of development the larva in its cocoon was a creamy yellow color and was distinctly segmented. During the early summer generation only one or two days elapsed before pupation. Larva maturing later in the summer overwintered in the prepupal stage. The adult wasp emerged from the cocoon by chewing an irregular hole near the anterior end of the cocoon.

The head widths of adult reared wasps were measured and associated with cell length. Data

EXPLANATION OF FIGURES 1–6

Developmental stages of *Dipogon sayi* on abdomen of spider host. Enlarged about 8 times. FIG. 1. Egg. FIGS. 2, 3, 4. Young larvae. FIGS. 5, 6. Nearly mature larvae.

[4]The writers wish to express their sincere appreciation to Dr. H. W. Levi for his helpful determination of the spiders.

1

2

3

4

5

6

TABLE I

EXAMPLES OF NEST CONSTRUCTION BY *D. sayi* IN HOLES OF ¼-INCH DIAMETER

NEST NO.	CELL NUMBER									INNER PLUG	VESTIBULE	OUTER PLUG
	1	2	3	4	5	6	7	8	9			
First Generation												
1	8†(w)									22	105	21
2	12♀	12†(s)								8	97	25
3	8P	9P	24♂							13	96	4
4	19†(w)	12♂	12†(w)							3	97	10
5	13♀	14♂	13†(w)	25P						4	38	26
6	20♀	12♀	13†(w)	12♀						19	56	15
7	14♀	8P	13†(w)	14P	12♀					none	none	72
8	8P	12P	8P	14*	12P					7	35	20
9	12♀	11♀	13♀	12♀	13P	34♀				3	40	3
10	10♀	11♀	18♀	15♀	12♀	21♂				3	45	9
11	12♀	10♀	13♀	11†(w)	10†(w)	8†(w)				5	38	10
Second Generation												
12	15♂	14P								10	151	9
13	7♂	7♀	6♂							14	118	7
14	4†(w)	6†(w)	7†(s)	11†(w)						9	91	7
15	5†(w)	10*	8†(w)	8♀	12†(s)					8	72	23
16	13♂	13†(w)	7♂	8♀	12†(w)					none	none	none
17	4♀	12♀	11♀	15♀	13♀	35♀				13	20	9
18	15♂	11♀	8♂	10♂	9†(s)	11P				4	85	7
19	13♀	8♂	8♂	12♀	9♂	8♂	90†(w)			none	none	none
20	10†(s)	12♂	9†(s)	8*	10†(s)	8♀	9†(s)	11†(s)	14*	18	17	3

The lengths of individual cells and outer plugs are given in millimeters, and the sex of reared wasps is indicated by ♂ and ♀. An asterisk (*) shows an improvisioned cell other than the vestibular cell: a parasitized cell is P, and † indicates that the wasp (w) died during rearing or the cell contained a dead spider (s).

TABLE II

HEAD WIDTHS OF ADULT WASPS (0.1 MM.) AND LENGTHS OF CELLS (MM.) FROM WHICH THEY WERE REARED IN ¼″ NESTS

SPECIMENS	HEAD WIDTH			CELL LENGTH			CORRELATION (r)
	Range	Mean	Standard Deviation	Range	Mean	Standard Deviation	
52 females	17–26	21 6	±2 24	4–37	13 6	±5 76	067 n.s.
35 males	12–18	15 0	±1 44	4–23	10 9	±4 52	198 n.s.

in Table II show that female wasps were larger than males, but that occasionally males were as large as small females. The cells containing female wasps averaged larger than cells with male wasps. However, considerable variation in length of cell was encountered in nests of this species, and no correlation between head widths of males or females with individual cells was shown.

Since the mother wasp provided only four cells on the average and was a fast builder, she ordinarily finished the nest in 1 or 2 days. Also, the life cycle of her progeny during the larval instars was only about one week, so that most nests contained cocoons when opened. However, among the large series of nests a few were found that contained young larvae; this enabled us to obtain some data on life history and make rearings at constant 15° and 27° C. temperatures.

Under field conditions the egg hatched in about 2 days and the larva was ready to spin its cocoon in another 5 to 6 days. We were not successful in exactly determining the number of molts in the larval cycle, but believe that five instars were present. The time from egg hatch to completion of cocooning was no more than 7 or 8 days. In the summer generation approximately 14 days were required for the pupal stage.

The development of the wasps was accelerated at 27° C., the larval stage being completed in 3 to 4 days, and the pupal stage in only 9 days. At 15° C., 14 to 17 days were required for the larval stage, and none pupated.

There was some competition between *D. sayi*, eumenid wasps, and megachiled bees for the nesting holes provided. Nests of the spider wasps superseded an eumenid nest four times, and a megachiled nest six times. A megachiled nest was found on top of *D. sayi* six times, whereas an eumenid nest was above only twice. It is not known how the various species would compete with one another in nature when only limited nesting holes were available, but the fact that mixed nests were found when adequate holes were available would suggest that strong competition for nest sites exists in nature.

The full grown larva of the second generation overwintered in its cocoon in diapause. Cocoons obtained from nests opened in the fall of 1955 were held at constant 4.5° C. temperature in order to determine the cold treatment required to break diapause. Random samples of cocoons were removed at intervals and held at 25° C. for rearing. Data given in Table III show that a

TABLE III

REARING OF WASPS AT 25° C. AFTER DIAPAUSING LARVA WERE HELD AT 4.5° C. FOR VARIOUS DAYS

NUMBER OF DAYS LARVAE HELD AT 4.5° C.	NUMBER OF ADULTS SUBSEQUENTLY REARED AT 25° C.	NUMBER OF LARVAE NOT REARED SUBSEQUENTLY AT 25° C.*
26	2	4
30	3	—
33	1	28
35	3	2
41	12	19
47	0	2
Total	21	55

*The experiments were started on Nov. 2nd. On April 18 all cocoons with unemerged wasps were opened. Most contained larvae that remained alive until the experiment was terminated on June 18.

few adult wasps were reared after 26 days of cold treatment, but that better results were obtained after 41 days. However, about two-thirds of the larvae did not pupate, and future experiments should be made at a wider temperature range and for different time periods in order to learn more about consistently breaking the diapause of the larva. Also, our data are approximations because the prior cold history of the cocoons in the field was not known. More information on cold treatments would be useful in establishing a better procedure for rearing the overwintering larvae in the laboratory.

The mature larvae and pupae of *D. sayi* were attacked by a chalcid parasite, *Melittobia chalybii* Ashm. The parasite larvae fed externally on their host in such numbers that only an empty shell or shriveled mummy of their host was left. In 28 examples where the host was totally consumed, 70.0±30.7, range 33–161, parasites were produced on a single larva. The parasite was particularly troublesome in our rearing studies, since the adult chalcid would enter vials by boring through the corks. Re-infestations were controlled by placing the small rearing vials in wide-mouth pint jars with screw caps. Because of the re-infestations experienced in the laboratory, we could not obtain accurate data on the number of cells which were parasitized. Probably not more than a very small percentage of the nests in nature would be parasitized, but the majority of larvae would probably be destroyed in each nest that was attacked successfully.

SUMMARY

More than two hundred nests of *D. sayi* were studied during the period 1952–1956. The nests were obtained almost entirely in sumac sticks with ¼ inch holes which had been placed in twenty localities in Wisconsin.

Considerable variation in the number of cells was encountered and there was no consistent pattern in the length of cell and sequence of sexes among cells. On the average, first generation nests consisted of three cells, second generation nests about five cells. Females were produced most frequently in the first-built cells, males in the last-built cells, but in some nests the sexes were mixed haphazardly.

Cells were provisioned invariably each with a single spider. Fourteen species of spiders in nine genera in four families were identified, but the most common were thomisid spiders in the genus *Xysticus*.

A detailed examination was made of 930 cells. Of these, 29 were unprovisioned and 170 contained dead or moldy spiders upon which an egg had either not been laid or had died. The contents of 553 cells were transferred to small vials and observed in the laboratory. Eighty-nine produced adults, 62 were parasitized by *Melittobia chalybii*, and the remaining 402 were discarded because of mortality and failure in rearing.

In nature, the life history of the first generation required a minimum of about 24 days. The second generation wasps overwintered as a full grown larva in a cocoon in a diapause. A minimum cold treatment of 26 days at 4.5° C. was required to break diapause, but a 41 day period of cold treatment was more effective.

D. sayi successfully competed with eumenid wasps and megachilid bees for nesting sites. In 16 mixed nests *D. sayi* superseded the other species 10 times.

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SPECIFICITY IN THE RESPONSE OF FEMALE TREE CRICKETS (ORTHOPTERA, GRYLLIDAE, OECANTHINAE) TO CALLING SONGS OF THE MALES[1]

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INTRODUCTION

A great many insect species possess specialized sound producing and receiving organs. Among these species are many which produce sounds loud enough to be noticed by man and which produce them continuously for many hours each day during their adult lives. On late summer evenings in many habitats in the eastern United States, there are twenty or more species stridulating at the same time, and the resulting din is astonishing to those unaccustomed to it. In spite of the loudness of the chorus and the fact that many of the chorusers are of economic importance, little is known about the biological significance of sound production in insects.

Loud persistent sound production is characteristic of the males of many species of Orthoptera. These males generally sing from the same place for extended periods. It is therefore supposed that the sounds attract females and result in copulation. An alternative or supplementary hypothesis is that the sounds result in spacing of the males.

If the songs of orthopteran males do cause the females to approach, a second supposition is that the sound of the male of one species attracts the females of that particular species and not those of others. That this is the case is supported by a considerable amount of circumstantial evidence. Alexander (1957) has discussed this evidence in detail, and a summary follows. First, many different species sing at the same time in the same habitat. If a female responded indiscriminantly to these sounds, she might go to several males of each of many species before she chanced upon one of her own species. This would have doubtful survival value. Second, the song of each species is characteristic of that species and different from those of all others in the same habitat. This generalization holds even for species that are so closely related as to be difficult to distinguish morphologically. Finally, there is ample evidence that the *males* of certain species of Orthoptera respond to sounds produced by their own sort and not to those of others. Several species synchronize or alternate their songs with the songs of others of the same species (Fulton, 1934) even though many species may be singing in the habitat. Males of some species group their songs so that there are periods of silence for that species followed by periods in which many males sing in close succession. Since specificity occurs among males, it is likely that it also occurs among females.

PREVIOUS WORK ON THE BEHAVIORAL EFFECTS OF ORTHOPTERAN SOUNDS

Experimental work as to the behavioral significance of Orthopteran sounds is scanty. Probably the earliest work was by Regen (1914), who demonstrated that the female of the cricket *Gryllus campestris* L. was attracted to the sound of the male. Regen controlled the factors of olfaction and vision by transmitting the sound of the male through a telephone. The female moved toward the sound emitting from the receiver. Duijm and van Oyen (1948) found that females of the shield-backed grasshopper *Ephippiger ephippiger* Fiebig were attracted to concealed singing males but not to males that had been silenced by gluing their wings together. Weih (1951) studied the acoustical behavior of seven species of Acrididae. He found that the species varied in their responses to the songs of other species. The males of most species would sing in alternation with males of other species, but alternation between male and female was more an intra-specific phenomenon.

With the advent of modern tape recording and high fidelity reproduction of sound, new possibilities were created for experiments dealing with the acoustical behavior of insects. Haskell (1953) played transcribed songs of the males of the cricket *Gryllus domesticus* L. to females of that species. He states that the "calling song" of the male initiates short bursts of locomotor activity, while the "courtship song" inhibits locomotor activity and results in a general passiveness of behavior.

Busnel and Busnel (1954) used modern equipment to demonstrate an attraction of the female of the tree cricket *Oecanthus pellucens* Scop. to the calling song of the male. In their experiments they either picked up the sound of a

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Special recognition is due George Potor, Jr., Division of Biophysics, Ohio State University, who generously contributed his time and skill in designing and constructing the setup which produced the artificial cricket sounds used in this study.

Thanks are given to Edward S. Thomas and Donald R. Meyer for reviewing the manuscript.

naturally singing male and transmitted it to the testing cage with appropriate high fidelity microphone, amplifier, and speaker or played a recording of the natural song to the female.

Busnel and Dumortier (1954), in work with *Ephippiger bitterensis* Chopard, showed that the female was not only attracted to the song of the male but also to artificial signals such as notes from a Galton whistle, certain bird calls, pure frequency signals, and square wave signals. Busnel, Dumortier, and Pasquinelly (1955) demonstrated that to induce a response, artificial signals must contain at least one "transient" (a sudden change in intensity).

Busnel, Busnel, and Dumortier (1956) experimented with the specificity of the songs of *Ephippigerida nigromarginata* Lucas and four species of *Ephippiger*. They found that males of each species responded to the sounds of males of the other species by singing in regular alternation or by changing the song rhythm. It is significant that none of these five species occur together in nature but were brought together for the purpose of the experiment. In experiments with *Ephippiger bitterensis* and *E. ephippiger*, females of *bitterensis* showed a more pronounced response to the song of a male of *ephippiger* than to the song of a male of *bitterensis*. This relationship was not entirely a matter of response to the song of highest intensity, nor was the response of the same nature as the response to artificial signals, since it occurred at lower intensities than the minimum intensity at which artificial sounds were effective. However, since these species do not occur together naturally, these experiments do not discredit the hypothesis that sound is an isolating factor among sympatric species.

Busnel and Loher (1954) studied the acoustical behavior of three acridids, *Chorthippus jucundus* Fisch., *C. brunneus* Thbg. (= *bicolor* Charp.), and *C. biguttulus* L. They found that artificial signals of a higher intensity than the normal song would induce responses in these species similar to the responses to natural sounds, *i.e.* alternation of song and, in *biguttulus*, phonotaxis on the part of the male. Busnel, Loher, and Pasquinelly (1954) showed that for these species a transient is an essential part of an effective artificial signal. Loher (1957) gives a complete account of his work with these species. He states that there is a greater specificity in the phono-responses of *biguttulus* than in the other two species. Perdeck (1955, 1957, unpublished data) worked with two of the above species, *brunneus* and *biguttulus*, and claims that "both male and female react exclusively to the song of conspecific partners. Hence, the described orienting and releasing effect of song is lacking between a male and a female of different species. . . ."

SINGING BEHAVIOR OF EASTERN TREE CRICKETS

The tree crickets (Oecanthinae) of eastern United States are wellsuited for a study of the specificity of response to sound in singing insects. They are abundant, and closely related species sing in the same habitat at the same time.

Among tree crickets only the males make noticeable sounds. These sounds are produced by specialized tegmina which during sound production are maintained in a plane nearly perpendicular to the axis of the body. The right tegmen overlaps the left. Near the base of the right tegmen and on its ventral surface is a vein called the file, which bears a series of teeth. Along the inside edge of the left wing is a sharp, heavily sclerotized section called the scraper. When the wings are moved mesad, the scraper engages the file, and the series of shocks produced by the striking of the teeth is transmitted to the wing membranes, causing them to vibrate. When the wings are returned to the starting position, the file and scraper do not engage and no sound is produced. When the male sings, the wings are opened and closed 15 to 100 times per second, each closure producing a *pulse* of sound of a rather pure pitch.

Male tree crickets produce sounds in several situations. The majority of the sounds are produced by solitary males which are not in contact with other individuals of the species. The song produced under these conditions is termed the *calling* song and induces a positive phonotaxis in sexually responsive females of the species. *Courtship* and *postcopulatory* songs are commonly produced when the male is in contact with a female of its species. The functions of these two types of songs have not been demonstrated, but they are assumed to stimulate the female to copulation and to continued feeding on the secretions of the metanotal glands of the male (see Jenson, 1909, for a description of behavior associated with copulation). Only the calling song will be discussed in the remainder of this paper.

The calling songs of the tree crickets of eastern United States are alike in that they consist of sequences of pulses delivered at a uniform rate. However, on the basis of the continuity of the pulse sequences, the species can be divided into three categories (fig. 1). (1) Species in which the pulse sequence continues without interruption for minutes at a time. This type of calling song will be referred to as a *continuous trill* and is characteristic of *Oecanthus latipennis* Riley, *O. pini* Beutenmuller, *O. nigricornis* F. Walker, *O. quadripunctatus* Beutenmuller, and *O. argentinus* Saussure. The last two species listed are usually classed as varieties of *O. nigricornis*, but the information presented in this paper indicates that they are distinct species. (2) Species in which the pulse sequence is discontinued and begun at irregular intervals of from one to several seconds. This type of calling song is designated a *broken trill* and is characteristic of

Oecanthus angustipennis Fitch, *O. exclamationis* Davis, and *Neoxabea bipunctata* (DeGeer). (3) Species in which the pulse sequence is broken into uniform bursts with uniform intervals. This type of calling song is called a ***regular chirp*** and is found in only one species of eastern tree cricket, *Oecanthus niveus* (DeGeer).

At any given temperature, the pulse rate of the song is characteristic for each species. However, with changes in temperature, there are corresponding changes in the pulse rate. Figure 2 shows the effect of temperature upon the pulse rates of each of the species studied. It is derived from the analysis of over 800 recordings made in the field and under controlled conditions in the laboratory. Most of the recordings were of

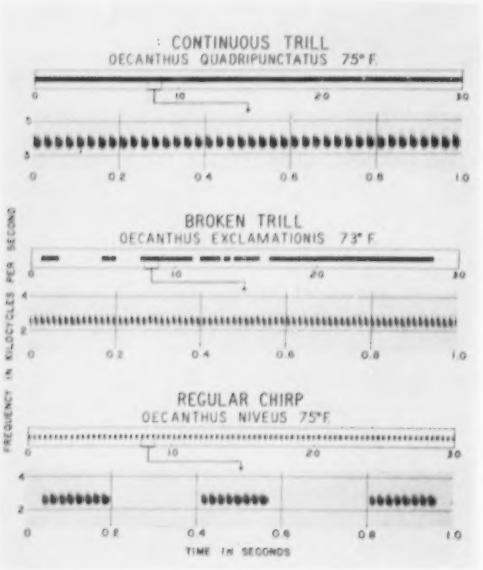

FIG. 1. Diagrams and audiospectrograms of the three types of calling songs. Note that the audiospectrograms show all calls to consist of sequences of pulses.

Ohio specimens, but recordings made in localities scattered throughout eastern United States (excepting New England) fall into the same pattern. The effect of temperature on each species appears linear, and there is only slight individual variation about the regression lines. In addition to a regular change in pulse rate with temperature, in *O. niveus* there is also a regular change in chirp rate. Since chirp rate is easily determined by counting the chirps in a specified time, it is possible to approximate the temperature by listening to a cricket of this species, and this characteristic has earned it the title of the "thermometer cricket" (see Allard, 1930, for details). Another change correlated with temperature is a change in the pitch of the song. In all species the songs become lower in pitch as the temperature decreases. A certain pitch is characteristic for each species at each temperature, and the range is from 1700 to 5200 cycles per second.

Humidity, light, and air currents do not affect the pulse rate or the chirp rate in tree crickets to any readily detectable extent (unpublished data).

The habitat in which each species ordinarily is found is indicated in figure 2. Species occurring in the same habitat at the same time usually have markedly different pulse rates. *O. nigricornis* and *O. quadripunctatus* are frequently found inter-

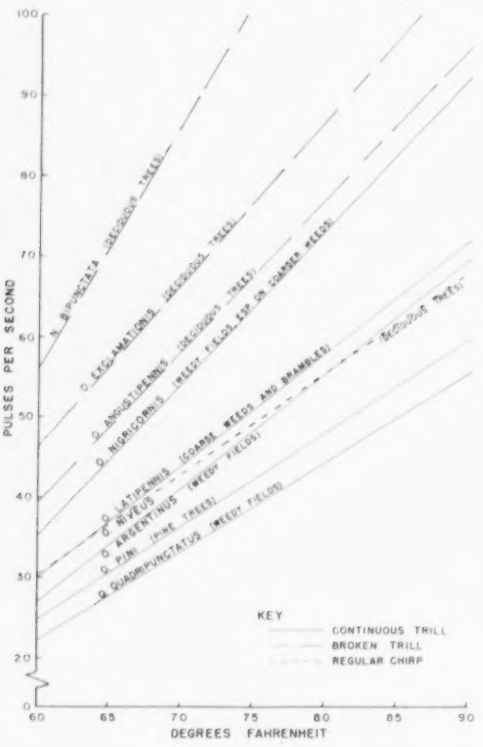

FIG. 2. Effect of temperature upon pulse rate in the calling songs of tree crickets.

mixed in large numbers in certain habitats in Ohio and Indiana, and the pulse rates of their songs are widely divergent and do not overlap. In some of the same habitats *argentinus* is also found, but it is to some extent isolated in time from the other two species. *O. argentinus* has two generations each summer in contrast to the one generation of *nigricornis* and *quadripunctatus*. The first generation of adults of *argentinus* disappears in early August at the time *nigricornis* and *quadripunctatus* begin to mature. The second generation of *argentinus* appears in mid-

September, and from then until frost adults of all three species may occur intermixed in the same habitat. The pulse rate of the song of *argentinus* never overlaps with that of *quadripunctatus* nor with that song of *nigricornis* illustrated in figure 2. However, recent work with Ohio specimens which are *nigricornis* on the basis of coloration has demonstrated that two song forms occur, the taxonomic status of which is undetermined. One form of *nigricornis* has the song illustrated in figure 2; the other has a slower song which is only slightly faster than that of *argentinus*. When the calling songs of 37 individuals were recorded under carefully controlled temperatures, 22 were found to be fast singers and 15 to be slow singers. No intermediate-type songs were recorded, and each individual produced only one of the two classes of songs. The males of the two song forms could ordinarily be separated morphologically on the basis of the number of teeth in the file. No other differences, either in morphology or biology, have been found. The

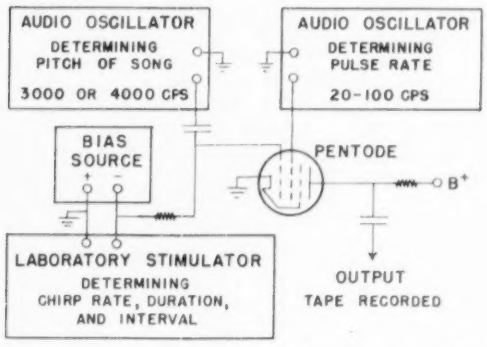

FIG. 3. Diagram of setup used to produce artificial calling songs.

females used in the experiments described in this paper were from a field in which the males were largely fast singers, and a check of the responses of the individual females showed no evidence that any of the females responded to the slower song rather than the faster one. Whether there are females which respond to the slower song is unknown.

Some interesting generalizations can be made concerning habitat, song type, and singing period of eastern tree crickets. The tree-dwelling forms sing almost exclusively at night. Forms occurring in open fields sing during both day and night; *O. latipennis* sings chiefly at night but is usually found along woodland edges. The three species with the highest pulse rates at a given temperature are the only ones which deliver broken trills, and all are tree-dwelling forms. Since they sing at night, when temperatures are low, their maximum pulse rates are not much higher than forms which sing at a lower rate at a given temperature but which sing during the heat of the day.

MATERIALS AND METHODS

The sounds used in these tests were of two origins—natural and artificial. Both types were first recorded on magnetic tape and then played back to the test insects under laboratory conditions. The natural sounds were calling songs of tree crickets usually recorded in the laboratory with a Magnecorder PT63-A tape recorder mechanism and a Magnecorder PT63-J recording and playback amplifier. Some of the recordings of natural sounds were made in the field with a Magnemite 610E recorder (Amplifier Corporation

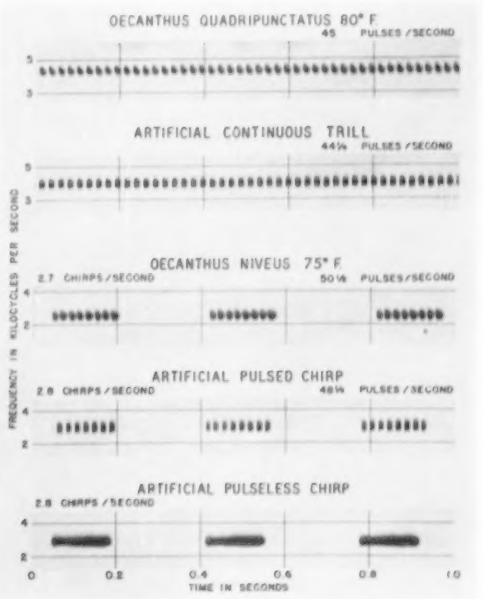

FIG. 4. Audiospectrograms comparing natural and artificial calling songs. The pulse rates given for *Oecanthus niveus* and its imitation are those within the individual chirps.

of America). In either case a dynamic microphone was used (Model D33 or D33A, American Microphone Company), and the tape speed was 15 inches per second.

Artificial tree cricket calls were produced by a device (fig. 3) designed and constructed by Dr. George Potor, Jr., Division of Biophysics, Ohio State University. The device was basically a pentode amplifier operating at 4000 (or 3000) cps, screen-grid modulated at 20–100 cps. This was grid biased by a variable DC souce to that portion of the plate-characteristic curve which allowed passage of bursts of 4000 (or 3000) cps signal. The grid bias could be further switched on and off by a laboratory stimulator to provide

chirps of variable frequency and duration. Pulseless chirps could be produced by cutting out the 20–100 cps audio oscillator. Figure 4 shows audiospectrograms of artificial tree cricket calls compared with genuine ones.

From the tape recordings of the signals to be tested, loops were made by splicing the ends of seven-foot sections of tape to their beginnings. In cases of recordings of regular chirps care was taken to join the tape at an interval between chirps and in such a manner as to maintain an unbroken chirp rhythm. The uniformity of the intervals between chirps was checked by making audiospectrograms with a Kay Electric Company Vibralyzer (Borror and Reese, 1953, give a short summary of the use of this machine in sound analysis). A loop of tape made in this manner, threaded into the Magnecorder and passed over a reel as shown in figure 5, could be played continuously for as long as an experiment required.

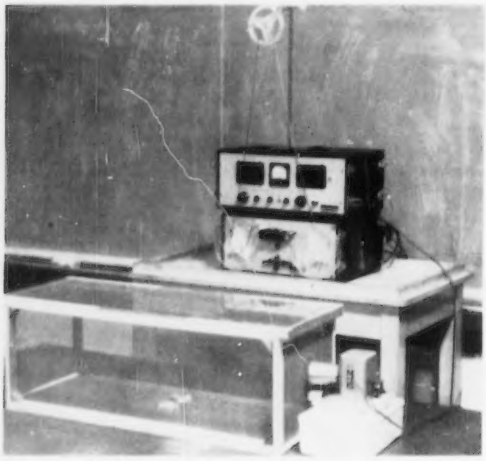

FIG. 5. Equipment used in testing the response of female tree crickets to recordings of artificial and natural sounds. The cage and speaker are in the foreground; behind them is the tape recorder with a loop of tape in place.

Each loop was marked to indicate the direction in which it should be played and was stored on a reel in a labeled envelope. The original loops are in the Library of Animal Sounds, Department fo Zoology and Entomology, Ohio State University.

The speaker used in these tests was a Jensen RP-302 "super-tweeter." It was mounted with a Jensen A-402 crossover network with a 15 ohm resistor taking care of the low-pass output. The intensity of the sound emitting from the speaker was standardized for each set of experiments with the VU meter of a Magnemite 610EV tape recorder (Amplifer Corporation of America). The original standard was that reading produced on the VU meter by a naturally singing tree cricket at one inch from the microphone. Thereafter, sounds to be tested were adjusted to give that same reading with the microphone one inch from the speaker. Later the Magnemite was calibrated with a General Radio Company Type 1551-A sound-level meter. At one inch from the outside edge of the speaker the maximum intensity was 93 db. Since the characteristics of the Magnemite as a sound-level meter vary with the voltage of its batteries, some variation from this intensity must have occurred during the several months of the tests. However, this Magnemite was seldom used except as a sound-level indicator, so the variation should not have been great. At any rate, for the tests carried out on any one night or on consecutive nights, the intensities were well standardized.

The tree crickets to be tested were confined in a cage (figs. 5 and 6) with inside dimensions 1′ x 1′ x 3′. The sides, bottom, and ends were made of

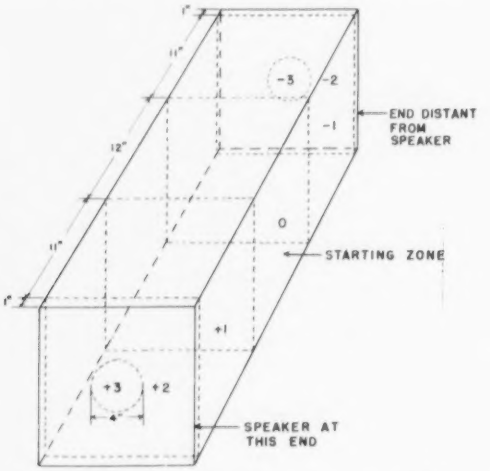

FIG. 6. Diagram of cage showing zones used in recording the positions of the crickets. The finely dashed lines indicate the boundaries of the zones, and the figures in the various zones represent the scoring system.

16-mesh plastic screening. The top consisted of three closely fitting panes of glass with the middle pane removable. The cage was constructed so that the supporting members were outside the glass and screening, leaving the inside surfaces free from obstructions. The acoustical properties of the cage were far from ideal, since standing waves were set up which caused an uneven intensity gradient from the speaker. The results of the tests indicate that the crickets were nevertheless able to orient to the sound source. In future work a cage of improved design should be used.

The crickets in these experiments were virgin females, obtained by rearing late-instar female nymphs to maturity in isolation. Virgin females

were used because they had no previous experience in responding to sounds and because they were more likely to be sexually receptive. All crickets used were from Franklin County, Ohio.

For each series of experiments the signals to be tested were selected, and two or three replicates were set up with the treatments randomized in each. At the beginning of each treatment ten virgin females of the test species were placed in the central section of the cage in a darkened room in which the temperature was held nearly constant (±1°F.). Previously the crickets had been marked with dots of colored paint on the pronota so that individuals could be recognized. The speaker was placed in the center of one end of the cage with the forward edge of the speaker one inch from the screening (fig. 5). From treatment to treatment the end at which the speaker was placed was randomized. The signal was played for ten minutes. At the end of this period a flashlight was used to determine the positions of the crickets in respect to the seven arbitary zones indicated in figure 6. If a cricket was on the line of division between two zones, it was considered as in the zone farther from the starting zone. After their positions were recorded, the crickets were returned to the starting position, and the next signal was played.

The results of the tests were evaluated in two ways—an arbitrary scoring system and a chi square analysis. The scoring system involved giving the seven cage zones used in recording the position of the crickets values of +3 to −3(fig. 6). With this system random movement on the part of the crickets would be expected to give an average score of 0. A maximum positive response for ten crickets would produce a score of about +25 and a corresponding negative response would be expected to yield a score of −25. The maximum score is estimated at 25 instead of 30, because ten crickets would not remain within a four-inch diameter circle; never were more than five observed within the circle at once. In the early stages of experimentation the +3 and −3 zones were not used in recording the positions of the crickets; therefore, the maximum score was +20. Before these scores were included in the tables of this paper, they were multiplied by 5/4 to make them more comparable with later scores.

Since the scoring system was arbitrary, the scores were not susceptible to ordinary tests of statistical significance. Therefore, a chi square test was also applied to the results. For this test the crickets which were outside the starting zone at the end of the test period were classed into two groups—***plus***, if they had moved in the direction of the speaker, and ***minus***, if they had moved away from it. If the movement was non-oriented, the plus group should be equal in number to the minus group. With the chi square test, the observed distribution could then be compared with the expected one to see if the observed deviated from the expected by more than could be accounted for on the basis of chance within certain probability limits. Since the chi square analysis takes into account only the direction of movement and not the extent, it does not utilize all the available information and is inferior to the scores as a basis of comparison between tests. It does, however, give an objective measure of the significance of the response in each test.

RESULTS

Specificity of Responses to Natural Sounds

The first problem investigated was whether female tree crickets are attracted to the calling songs of other species occurring in the same habitat. Table I shows the results of experiments with females of *O. quadripunctatus* and *O. nigricornis*, two species which occur in weedy fields. Table II shows the results of experiments with females of *O. niveus* and *O. exclamationis*, two species which live in trees. In each case a positive phonotaxis by the females to the calling songs of the male of the same species was demonstrated. No such responses were demonstrated to calling songs of other species occurring in the same habitats. It is of course possible that some response existed, but that it was too small to be demonstrated by the technique used. In any event there can be no doubt that female tree crickets show a much more pronounced positive response to calling songs of their own males than to those of males of other species singing in the same habitat.

Basis of Specificity among Species Producing Continuous Trills

If female tree crickets respond to the calling songs of their own species and not to similar songs of other species in the same habitat, the question arises as to what is the basis of this specificity. The continuous trill, the simplest type of song studied, was the first to be investigated to determine what component was responsible for differential responses among females of different species.

A study of the calling songs of species producing continuous trills reveals that pulse rate is the characteristic which differs most widely among species occurring in the same habitat. Thus it seems a logical hypothesis that the female's discrimination is based on a response to certain pulse rates. The functioning of insect tympanal organs supports this contention, since their response depends on the pattern of sound intensity changes (amplitude modulation pattern) so long as the carrier frequency is within the range audible to the insect (Pumphrey, 1940). The pulses in a tree cricket trill are just such amplitude modulations.

The hypothesis that pulse rate is the song

TABLE I

RESPONSE SCORES AND CHI SQUARE VALUES RELATING TO SPECIFICITY OF RESPONSE IN FEMALES OF SPECIES FOUND IN WEEDY FIELDS

	Recordings Tested		
	O. quadripunctatus at 74°F. 37¾ pulses/sec. 3600 cycles/sec.	*O. argentinus* at 74°F. 44½ pulses/sec. 3500 cycles/sec.	*O. nigricornis* at 75°F. 64¼ pulses/sec. 3700 cycles/sec.
O. quadripunctatus females, 74°F.			
Replicate 1	+19	+4	——
Replicate 2	+21	+1	——
Average	+20	+2	——
Chi Square	10.00*	0.82	——
O. nigricornis females, 75°F.			
Replicate 1	+1	+3	+17
Replicate 2	+2	+2	+19
Replicate 3	−3	0	+15
Average	0	+2	+17
Chi Square	0.25	1.29	19.59*

*Significant at the 1% level.

TABLE II

RESPONSE SCORES AND CHI SQUARE VALUES RELATING TO SPECIFICITY OF RESPONSE IN FEMALES OF TREE-DWELLING SPECIES

	Recordings Tested		
	O. niveus at 75°F. 2.7 chirps/sec. 50½ pulses/sec. 2600 cycles/sec.	*O. angustipennis* at 74°F. 53 pulses/sec. 2600 cycles/sec.	*O. exclamationis* at 76°F. 80 pulses/sec. 2600 cycles/sec.
O. niveus females, 75°F.			
Replicate 1	+10	+4	+3
Replicate 2	+10	+4	+1
Replicate 3	+14	0	−1
Average	+11	+3	+1
Chi Square	10.70*	1.92	0.20
O. exclamationis females, 75°F.			
Replicate 1	+1	−2	+6
Replicate 2	0	+5	+12
Replicate 3	−1	−3	+6
Average	0	0	+8
Chi Square	0.00	0.17	7.36*

*Significant at the 1% level.

component resulting in specificity of response is complicated by the fact that the pulse rate for each species varies with temperature. Different species sing at identical pulse rates if their temperatures are properly adjusted. Therefore, if pulse rate is the basis for specificity of response of the female, the female must respond to different pulse rates at different temperatures.

To test the role of pulse rate in the response of females of a species, two sorts of recordings were used: (1) recordings of songs of the species made at different temperatures and therefore having different pulse rates and pitch and (2) recordings of artificially produced trills having different pulse rates but the same pitch. Table III shows the results of an experiment of this nature with females of *O. quadripunctatus*. It is apparent that the crickets responded to markedly different degrees to the same signals at different temperatures. At 70°F. there was a statistically significant response to the calling song of the male at 70°F. and to an artificial trill of 32½ pulses per second; there was no such response to the song of the male at 80°F. or to an artificial trill of 44½

TABLE III

RESPONSE SCORES AND CHI SQUARE VALUES SHOWING THE EFFECT OF TEMPERATURE ON THE RESPONSE OF FEMALES OF *O. quadripunctatus* TO CERTAIN SOUNDS

	Recordings Tested			
	O. quadripunctatus		Artificial Trill	
	at 70°F. 33¾ pul/sec. 3600 cyc/sec.	at 80°F. 45 pul/sec. 4200 cyc/sec.	32½ pul/sec. 4000 cyc/sec.	44¼ pul/sec. 4000 cyc/sec.
O. quadripunctatus females, 70°F., 22 Aug. 1956				
Replicate 1	+14	+2	+18	−1
Replicate 2	+21	+8	+12	+7
Average	+18	+5	+15	+3
Chi Square	9.31[a]	1.00	8.33[a]	0.11
O. quadripunctatus females, 80°F., 23 Aug. 1956				
Replicate 1	−3	+18	0	+21
Replicate 2	+11	+20	−1	+12
Average	+4	+19	0	+16
Chi Square	0.50	9.00[a]	1.00	11.00[a]

[a]Significant at the 1% level.

pulses per second. At 80°F. the situation was reversed, with the females showing a response to the last two signals but not to the first two. Any role of pitch in the specificity of response seems to be eliminated by the responses to artificial trills of controlled pitch.

In a test of 74°F., again with *O. quadripunctatus* females, a positive phonotaxis was demonstrated to an artificial trill of 36½ pulses per second but not to an artificial trill of 44¼ pulses per second. These pulse rates correspond to those of males of *O. quadripunctatus* and *O. argentinus* at that temperature. This test substantiates the importance of pulse rate in explaining the data for *quadripunctatus* females in Table I.

In order to determine the extent to which females of a species respond to different pulse rates at given temperatures, *O. nigricornis* females were tested at 70° and 80°F. with artificial trills of varying pulse rates. Figure 7 shows the results of these tests. Each point on the graph represents two tests (using ten crickets each). Although the data are insufficient to give a smooth curve of response at each temperature, it is obvious that the ability to respond to given pulse rates varies with temperature and that the pulse rate producing maximum response at a given temperature approximates that produced by the male of the species at that temperature. It is also evident that at a given temperature there is a range of pulse rates to which females of a species will respond.

Before pulse rate can be singled out as the sole factor resulting in specificity of response in species producing continuous trills, another possibility must be evaluated—the possibility of the occurrence of elements in the song of one species that

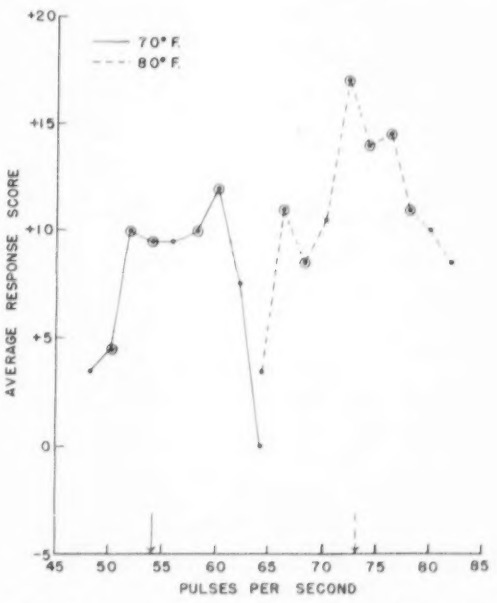

FIG. 7. Response of *Oecanthus nigricornis* females at 70° and 80°F. to artificial, 4000 cps continuous trills of different pulse rates. The encircled points are those for which chi square analysis showed a response significant at the 1% level. The arrows on the lower scale indicate the average pulse rates of the songs of the males at the two temperatures.

inhibit or repulse the females of other species. This possibility is easily checked by selecting recordings of different species singing at the same pulse rate (and hence at different temperatures) and seeing whether females of one species will respond to all songs. This was done using *O. nigricornis* females at 70°F. and recordings of *argentinus* and *quadripunctatus* at pulse rates similar to *nigricornis* at 70°F. The results are listed in Table IV. The responses of the *nigricornis* females to the songs of all three species were nearly equal, so there seems to be no inhibitory element in the song of one species to the females of another. The results of tests with the speaker turned off are given in Table IV to illustrate scores produced by movement without relation to sound. In other tests with no sound, scores as high as +4 were obtained.

Specificity in Species Producing Broken Trills

Among species with broken trills, pulse rate varies widely, and one would suspect it to be the basis for specificity of response. However, another factor which might be of importance in inducing a positive phonotaxis in the females of these species is the occurrence of discontinuities in the song. To test the effect of this latter factor, females of *O. exclamationis* were played both broken and continuous trills of their own species. A continuous trill was produced by making a loop from a recording of a pulse sequence over five seconds in length.

The results are shown in Table V, and it is apparent that the females responded at least as well to a continuous trill as to a broken trill. The data of a further test shown in Table V indicate that *O. exclamationis* females respond positively to an artificial trill of the same pulse rate as the natural song, even though the pitch is quite different. It therefore seems that the factor responsible for specificity in species producing broken trills is the same as in species producing continuous trills, *i.e.* pulse rate.

Specificity in O. niveus

In the song of *O. niveus* there are two regular rhythms—the pulse rhythm and the chirp rhythm. It is conceivable that either pulse rhythm or chirp rhythm alone induces positive phonotaxis in the female. Since the two are inseparable in the natural song of *niveus*, artificially produced signals were used to test whether one or both were responsible. Three artificial signals were played to females at 75°F.: (1) continuous trill of 48½ pulses per second (pulse rate similar to natural *niveus* but no chirp), (2) regular, pulseless chirp at 2.8 chirps per second (chirp rate similar to natural *niveus* but no pulse), (3) regular, pulsed chirp at 48¼ pulses per second and 2.8 chirps per second. The pitch was imperfectly controlled—4000 cps in (1) and 3000 cps in (2) and (3)—because more suitable recordings were not available at the time. However, in work with other species there was no indication of a role of frequency differences within this range. For instance, *O. exclamationis* females responded as well to an artificial trill pitched at 4000 cps as they did to the natural trill at 2600 cps (Table IV).

Table VI shows that the crickets did not respond significantly to the continuous trill but showed a positive phonotaxis to the pulseless chirp. This indicates that chirp rhythm is essential in the response of the female of *O. niveus*. Since pulsed chirps produced more pronounced phonotaxis than pulseless chirps, the pulse rhythm must have a supplementary effect. The prime importance of the chirp rhythm makes the response quite specific, since *O. niveus* is the only chirper with a uniform rhythm in its habitat.

The range of chirp rates to which *niveus* will respond at a given temperature was not determined. In eastern United States there is no species with a similar song, so there is no selective advantage in a narrow range of response. In western United States there is a tree cricket with a slower chirp, but it occurs in a different habitat.

DISCUSSION

These experiments demonstrate that insect-produced sounds may be important in the reproductive isolation of sound-producing species. Minor differences in the rhythm or rate of song may determine whether or not the female of a singing species responds to a song. If song is important in species isolation, it can play a major role in the evolution of new species. Once song differences have developed and become behaviorally significant, sibling sound-producing species can live in close contact with reduced chances of hybridization. The songs of closely related species occurring in the same habitat can be expected to differ to a greater extent than characters which do not influence the maintenance of species isolation. Most morphological characters used in classifying the Orthoptera are not directly related to the maintenance of reproductive isolation, so studies of song differences can be expected to clear up many taxonomic problems concerning sound-producing species (Fulton, 1933; Alexander, 1957a).

An example of the value of song in taxonomy is the clarification of the status of *O. quadripunctatus* and *argentinus* as species distinct from *O. nigricornis*. As pointed out above, these three species have distinctive songs, and the females are specific in their phono-responses. Other differences, in life history and morphology, exist and will be described in a later paper.

The study of the behavioral significance of insect-produced sounds is a field which has been

TABLE IV

RESPONSE SCORES AND CHI SQUARE VALUES SHOWING THE RESPONSE OF *O. nigricornis* FEMALES TO THE SONGS OF THREE SPECIES SINGING AT SIMILAR PULSE RATES

	Recordings Tested			
	O. nigricornis at 70°F. 53 pulses/sec. 3300 cyc/sec.	*O. argentinus* at 80°F. 56¼ pulses/sec. 3900 cyc/sec.	*O. quadripunctatus* at 89°F. 55¼ pulses/sec. 4400 cyc/sec.	No Sound
O. nigricornis females, 70°F.				
Replicate 1	+14	+11	+12	−1
Replicate 2	+9	+16	+10	−1
Average	+12	+14	+11	−1
Chi Square	8.33[a]	10.89[a]	8.00[a]	0.33

[a]Significant at the 1% level.

TABLE V

RESPONSE SCORES AND CHI SQUARE VALUES SHOWING THE RESPONSE OF *O. exclamationis* FEMALES AT 76°F. TO CONTINUOUS AND BROKEN TRILLS

	Recordings Tested		
	O. exclamationis at 76°F. 80 pulses/sec., 2600 cycles/sec.		Artificial Trill 80 pulses/sec. 4000 cycles/sec. (continuous)
	Broken Trill Bursts of 2–2½ sec. Intervals of ¼–½ sec.	Continuous Trill	
Replicate 1	+16	+17	+19
Replicate 2	+15	+18	+17
Average	+16	+18	+18
Chi Square	9.00[a]	8.00[a]	9.00[a]

[a]Significant at the 1% level.

TABLE VI

RESPONSE SCORES AND CHI SQUARE VALUES SHOWING THE RESPONSE OF *O. niveus* FEMALES AT 75°F. TO CERTAIN ARTIFICIAL AND NATURAL SOUNDS

	Recordings Tested			
	Artificial Trill 48¼ pulses/sec. 4000 cycles/sec.	Artificial Pulseless Chirp 2.8 chirps/sec. 3000 cycles/sec.	Artificial Pulsed Chirp 48¼ pulses/sec. 2.8 chirps/sec. 3000 cycles/sec.	*O. niveus* at 75°F. 50½ pul/sec. 2.7 chirps/sec. 2600 cyc/sec.
Replicate 1	+6	+5	+20	+16
Replicate 2	−1	+15	+20	+20
Average	+2	+10	+20	+18
Chi Square	0.40	5.40[b]	16.00[a]	16.00[a]

[a]Significant at the 1% level.
[b]Significant at the 5% level.

neglected and promises interesting discoveries. The calling songs of tree crickets are very simple compared with those of many other species of Orthoptera. In some species different kinds of sound pulses are produced, and there are gradual and sudden changes in rhythm, rate, and intensity. Yet the sequence and nature of these changes are constant for the species. The significance of the different elements of these complex songs should be explored. Effects of songs produced by males in contact with other males or with females offer an additional field for study.

SUMMARY

1. There is circumstantial evidence that the females of sound-producing Orthoptera respond to the songs of males of their own species and not to those of others; however, experimental evidence in scanty.

2. The calling songs of nine species of eastern tree crickets (Oecanthinae) were studied and found to consist of pulses of an almost pure pitch delivered at a uniform rate. On the basis of the continuity of the pulse sequences, the species were separated into these three groups: song a continuous trill, song a broken trill, song a regular chirp. In each species the pulse rate of the song varied directly with temperature. At any given temperature, the songs of species commonly found together had different pulse rates.

3. Experiments in which caged virgin females of four species of tree crickets were subjected to recorded natural and artificial sounds suggest these generalizations:

(1) Sexually responsive females show a positive phonotaxis to the calling songs of the males of their own species but not to the songs of other species occurring in the same habitat.

(2) In species with continuous trills, pulse rate is the determining factor in the response of the female to the calling song. The specific pulse rate to which the female exhibits greatest response varies with temperature in the same manner as does the pulse rate in the song of the male.

(3) In species with broken trills, females are equally attracted to continuous trills of the same pulse rate, and pulse rate is still the determining factor in the response of the female.

(4) In species with regular chirps, the pulse rate does not determine the response of the females, since they will respond to pulseless chirps. The response is to a regular series of sounds of longer duration than the pulses. However, the pulse rhythm may strengthen the response of females to the chirp rhythm.

4. These experiments support the contention that song is directly related to reproductive isolation among singing insects. If so, it should be of major importance in the evolution of species. The songs of closely related species occurring together can be expected to diverge more rapidly than characters which are unrelated to reproductive isolation—such as many of the morphological characters used in taxonomy.

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THE USE OF PESTICIDES IN THE PREPARATION OF INSECTS AND MITES FOR PHOTOGRAPHY[1]

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In the course of laboratory investigations on the comparative effectiveness of various acaricides against the two-spotted mite, *Tetranychus telarius* Linn., the writers (Ebeling and Pence, 1953) noted that, when treated with Aramite or Chlorobenzilate, the mites, after they are killed by the treatment, retain a remarkably lifelike appearance and posture (fig. 1A, left). Even the legs are automatically arranged in a symmetrical manner, much as the legs of the larger insects are arranged by the curator on specimens carefully prepared for permanent collections or exhibits. Other acaricides left the mites in varying degrees of disarrangement. The organic phosphates had a particularly striking effect in rapidly changing the appearance of the mites. Even before they were dead, and while they were still crawling about, the mites became shrunken and dark in appearance, and when dead they were very shrivelled (fig. 1A, right).

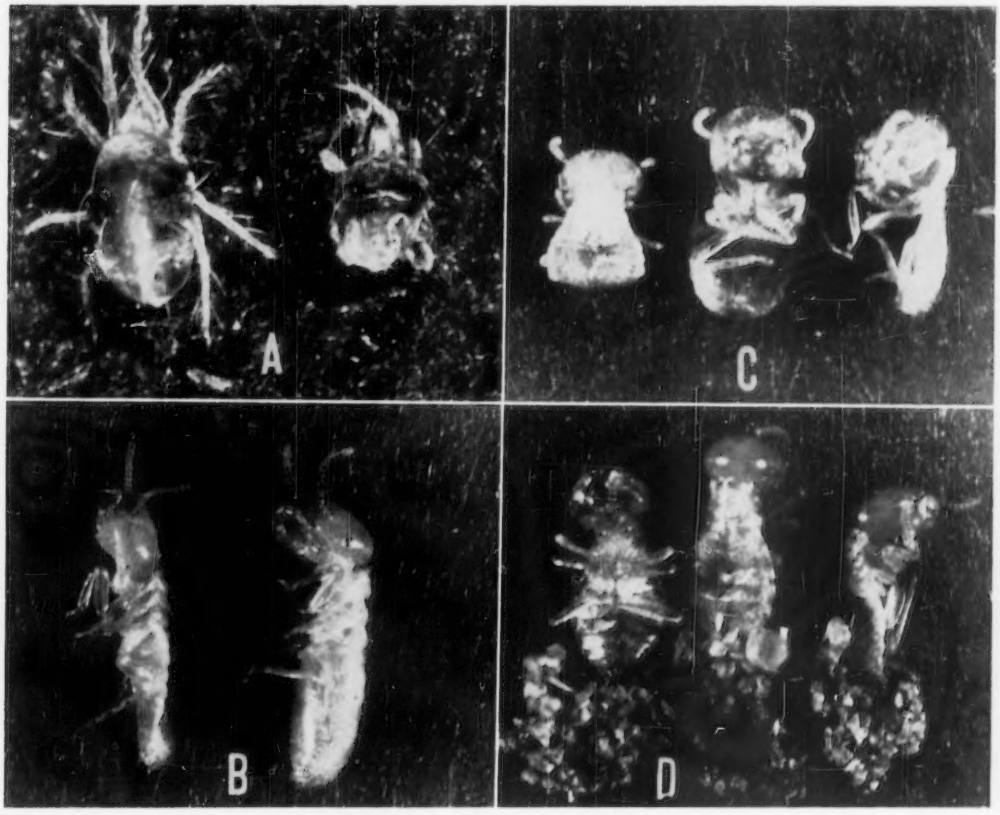

FIG. 1. The appearance of mites and insects killed by various pesticides. A, mites treated with Chlorobenzilate (left) and parathion (right); B, termites treated with parathion (left) and pentachlorophenol (right); C, termites treated with sodium fluoride, showing greatly flattened and distorted bodies; D, termites treated with sodium fluoride, showing attached feces and sand particles.

The various degrees of desiccation resulting from the different acaricides appears to indicate varying degrees of effect from these compounds on the water-permeability of the cuticle.

In experiments with the western subterranean termite, *Reticulitermes hesperus* Banks, penta-

[1]Accepted for publication April 4, 1957.

chlorophenol and sodium pentachlorophenate were found to have the same effect in preserving a lifelike appearance and posture of the termites (fig. 1B, right), as Aramite and Chlorobenzilate had when applied to mites. At least in part, this may be attributed to the tendency of the insects to die soon after the beginning of paralysis. Other insecticides may paralyze the insects much more quickly, but they may remain in a paralyzed

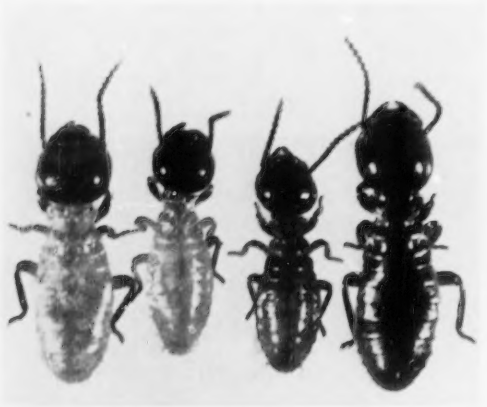

FIG. 2. Progressive discoloration of the bodies of termites killed by sodium pentachlorophenate solution. First the head, antennae, and legs, and finally the entire body, become shiny black.

state for days before dying. The organic phosphates again proved to have a marked effect in disarranging the appendages and changing the form and appearance of the treated subjects (fig. 1B, left). They had a desiccating effect on the termites and left them flattened dorsoventrally. However, the effect of sodium fluoride was even greater in this respect, apparently being accentuated by excessive defecation. The termites were flattened, curled up, and desiccated to an extreme degree (fig. 1, C and D). The chlorinated hydrocarbons caused the termites to have a wet appearance and they had the tendency to adhere to one another in masses.

A phenomenon that was unique to the pentachlorophenol- or sodium pentachlorophenate-treated termites was the black discoloration, first of the head, antennae and legs, and then of the remainder of the body, a day or two after the death of the termites. Progressive stages of this discoloration are shown in figure 2.

In the entomology laboratory of the University of California, Los Angeles, the usual technique for the preparation of insects for photography is to anesthetize them with carbon dioxide and photograph them at the moment of recovery when they have a lifelike appearance, but before they are sufficiently recovered to crawl or fly away. However, a treatment that can preserve them in a lifelike appearance and posture has obvious advantages when it is desired to place them in a certain position or posture and in a definite relationship to other objects, as in an *in situ* photograph. Likewise it is indispensable for the photography of insects or mites arranged in groups, particularly when different life stages, different castes, or different species, that vary in their response to carbon dioxide, are placed in the same group.

Experiments with other species and with a greater variety of chemicals might reveal other compounds that would be advantageous for use in killing insects or mites for photographic purposes.

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IMPORTANT NOTICE TO AUTHORS

In accordance with action taken by the Editorial Board, each manuscript submitted for publication in the ANNALS OF THE ENTOMOLOGICAL SOCIETY OF AMERICA after September 1, 1957, must be accompanied by an abstract prepared in accordance with the requirements of Biological Abstracts, and typewritten (double spaced) *on a separate sheet* (or sheets) of paper. The abstract will replace the usual author's summary. A more detailed announcement of this requirement appears on page 1 in the Bulletin of the Entomological Society of America Vol. 3, June, 1957.

THE LARVA OF CYPHOMYIA (DIPTERA, STRATIOMYIDAE) AND ITS SIGNIFICANCE IN CLASSIFICATION[1]

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Cyphomyia and related genera have been placed in the Stratiomyinae on the basis of adult characters, chiefly wing venation and abdominal segmentation, despite the fact that these flies are clearly more primitive than the *Stratiomys-Eulalia* branch. James (1940) proposed the tribal name Cyphomyini for this complex. However, the previously undescribed larva is so greatly different from the known Stratiomyinae, both in structure and in habits, that it can not be considered as belonging to the same subfamily. Adult differences, likewise, assume greater importance when considered in conjunction with these larval differences. I am, therefore, raising the tribe Cyphomyini to subfamily level.

Cyphomyiinae, new subfamily

Cyphomyini (tribe), James, 1940, Rev. de Ent., 11: 120; James and Steyskal, 1952, Ann. Ent. Soc. Amer., 45: 386. Type of subfamily, *Cyphomyia* Hoffmannsegg, in Wiedemann, 1819; based on *C. auriflamma* Hoffmannsegg.

Cyphomyia pilosissima Gerstaecker

1857, Linnaea Ent. 11: 292; Williston, Biol. Centr. Amer. I, p. 244; James, 1939, Proc. U. S. Nat. Mus., 86:601; James, 1940 Rev. de Ent., 11: 145.

Mature larva. Length, 18 mm. General color reddish brown (specimens preserved in alcohol), with paler markings.

Head (fig. 1) slightly longer than maximum width, tapering from eyes forward to the blunt snout. Frontal stripe smooth but not shining, not clearly differentiated except in color and only slightly elevated, not separated from the labrum. Antennae minute, set far back from apex; eyes large; two bulbous tubercles behind each eye. Labium very slender and elongated, four times as long as its maximum width, shaped like an attenuated hourglass, inserted at about the basal fourth of the ventral surface of the head and reaching the maxillary brushes, the lower part more heavily sclerotized and light brown, the apical part softer and whitish. Chaetotaxy of head consisting of two pairs of bristles on the frontal stripe, the anterior one the larger, and two small, closely set setae on the labrum; a large bristle above and a similar one below each eye, and a smaller bristle, in line with these, between the eye and the labium; two small to medium-sized bristles on each side of the oral margin, one just opposite and the other just behind the apex of the labium; and a small bristle below and a medium one anterior to each antenna. Frons, labrum, eyes, postocular tubercles, area around antenna and most of ventral surface yellowish white; darker areas of head shagreened, the integument beset with hexagonal plates; on the ventral surface, these plates white, outlined with reddish-brown, thus producing a dilute coloration.

Body outline oval, the sides of the individual segments approximately straight, only moderately indented at the incisures. Measurements in micrometer units (30 = 1 mm.), approximate only, because of the flexibility of the integument: head length 54, maximum width 51; prothorax length 38, width at anterior margin 77, at posterior margin 87; maximum width of mesothorax 117, of metathorax 138, of first abdominal 144, of abdomen (third segment) 151, of seventh abdominal 138; length of segments from mesothorax through seventh abdominal varying from 40 to 50; width of eighth abdominal 105 at base, 63 at its truncated apex; length of eighth segment 65. Thoracic spiracle large, lateral in position, with two evident slits (fig. 3). Small larval spiracles present on abdominal segments 1 through 7; a small, crescentic pupal spiracle[2] arching around a calcareous plate and lying above a crescent-shaped row of three to six calcareous plates, on each of abdominal segments 2 to 6 inclusively (fig. 2). Cleft of posterior spiracle visible on dorsal surface, near apex (fig. 4).

Integument of body pale reddish brown in ground color, thickly beset with small roundish to hexagonal whitish calcareous plates and with areas of brighter reddish brown appressed scales, the individual scales being smaller than the calcareous plates and usually thickly set. Dorsally, the anterior and posterior margins of the thoracic and of the first seven abdominal segments somewhat elevated with low transverse ridges occupying approximately the anterior and posterior fourth or fifth of the segment, these ridges appearing more whitish because of the greater prominence of the calcareous plates, except where obscured by the scaly areas; ventrally these areas but very slightly elevated; the conjunctival incisures prominent. Anterior margins of thoracic and of first abdominal terga beset with scales, the calcareous plates being almost obscured; narrow rows of these scales present on the anterior margins of abdominal terga 2 to 7 and on the posterior margins of the third thoracic and first seven abdominal terga, the tergal scales limited to the raised margins; no scales evident on the

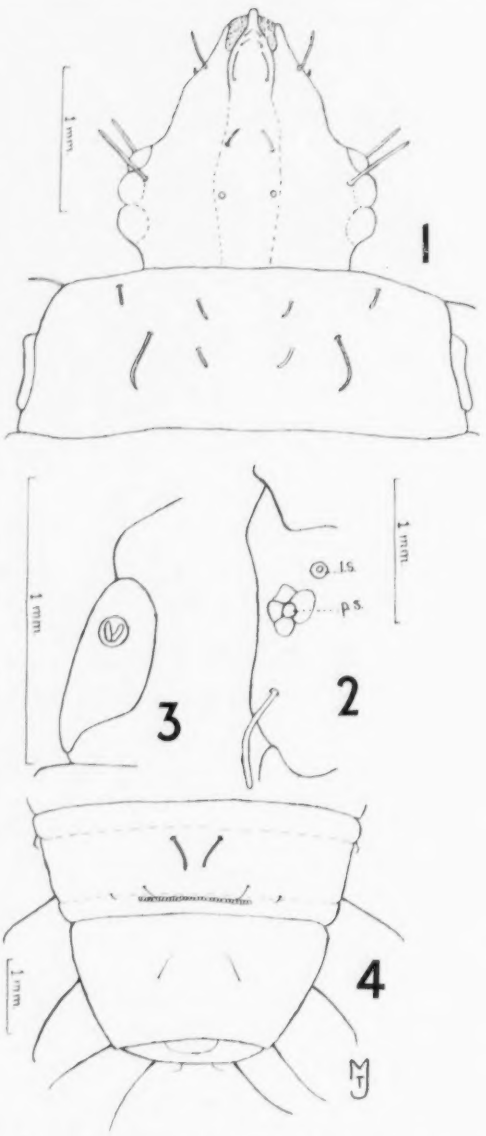
[1]Accepted for publication, May 15, 1957.

[2]This term is used, for want of a better one, to designate the areas where the pupal respiratory horns will develope. These horns are quite distinct in the puparium.

last tergum. Ventrally, these scaly areas in a similar pattern but more extensive; in addition, a median patch on each sternum beginning with the second thoracic, where it is small, increasing in size to the third abdominal tergum, where it takes in almost the median third from anterior to posterior margin, and continuing so to the seventh; eighth sternum beset with scales on the crescent-shaped perianal ridge and along the anal margins. Sixth sternite with a small oval whitish spot, in its center and extending lengthwise, composed of very closely packed, minute plates, each about half the diameter of the ordinary calcareous plates; this spot about one-third as long as the length of the segment.

Dorsal chaetotaxy of thorax and first seven abdominal segments consisting of three pairs of bristles per segment (figs. 1, 4), with an accessory median pair on the prothorax; the outer pair always small, located near the anterior margin on the thoracic and near the posterior margin on the abdominal terga; the intermediate pair large and strong on the thorax, where it occupies a median position along the longitudinal axis of the segments, but suddenly becoming weak and posteriorly located on the abdomen; the innermost pair always occupying a median position on the longitudinal axis, weak on the thorax but suddenly becomes strong on the abdomen. Laterally, a weak, curved bristle near the anterior margin of each segment except the last abdominal, and a strong bristle on each segment except the prothorax; this bristle is median in longitudinal axis on the mesothorax and gradually moves posteriad, being almost at the apical margin of the eighth segment, which also bears a strong median lateral pair, a strong apical pair, and a pair of weak, curved bristles between the apicals (fig. 4). Thorax ventrally on each segment, with a moderately small bristle near the anterolateral margin, a strong double bristle on each side of the median half, near the middle of the segment on the lengthwise axis, and a minute pair between these; chaetotaxy of first seven abdominal segments similar, except that each double bristle is replaced by a well-separated pair; eighth abdominal sternum with eight strong bristles, two arranged transversely to each perianal ridge and one on each side of the perianal ridge.

Described from two larvae and one puparium, with associated reared adults, Tlaycala, Mexico, K-66 (Puebla Highway), Coll. Dec. 1, 1956, preserved Feb. 26, 1957, ooze in base of maguey; W. E. Snow. Associated adult males and females from the same collection emerged on Jan. 29 and Feb. 22, 1957.



Cyphomyia pilosissima Gerstaecker, mature larva. FIG. 1.—Head and prothorax, dorsal view. FIG. 2.—Prothoracic spiracle, dorsolateral view. FIG. 3.—Side of third abdominal segment, showing larval spiracle (l.s.) and site of pupal spiracle (p.s.). Fig. 4.—Seventh and eighth abdominal segments, dorsal view.

DISCUSSION

In addition to the larvae and puparium described above, I have examined an empty puparium of *Cyphomyia marshalli* Lindner, from the Solomon Islands, and a drawing of the larva made by Dr. Clifford Berg[3]; two puparia, in poor condition, of *C. bicarinata* Williston, from Tucson, Arizona; and a series of undetermined

[3]To be described in a forthcoming paper by Dr. Berg. The puparia of *C. bicarinata* are not in good enough condition to warrant description at this time.

larvae from Honduras. This is admittedly a meager representation of a group which contains about a hundred species, but, though differing in some quite obvious characters of specific importance, these four agree in the possession of a set of outstanding characters which would appear to be of generic value. These are summarized as follows.

Head large, conical to elongated-conical; frontal stripe distinctly demarcated; antennae distinct though very small, two-segmented, lateral in position, removed some distance from the head apex; eyes prominent, with two or more tubercles behind and sometimes also below each. Prothoracic spiracle set on a large tubercle, laterally located; spiracle with two slits which diverge into a V opening anteriorly; a small, anterolaterally located, inconspicuous spiracle present on each of the first seven abdominal and sometimes on the metathoracic terga; sites of pupal respiratory horns (visible only as the mature larva approaches pupation) present anterolaterally on abdominal segments 2, 3, 4, and 5; spiracular slit of last abdominal segment dorsal, not fringed with hairs. Bristles of thorax and abdomen arranged in three longitudinal rows on each side of the median line; those of the innermost row weak on the thorax, strong on the abdomen; those of the intermediate row either all strong or else stronger on the thorax; those of the outer row weak; the bristles either simple or pubescent (sp. from Honduras); the ventral segments, on the abdomen, each with two well spaced bristles, in a transverse row, on each side of the median line; on the thorax, these bristles united into a double bristle on each side.

The larvae of the Stratiomyinae (including the undescribed larva of ***Myxosargus nigricornis*** Greene, which I have examined) differ in the smaller, less tapering head; the relatively large antennae, which are usually more anterior in position; the less well developed eyes and the lack of tubercles behind and below them; the dorsally placed prothoracic spiracles, which lack definite slits and which are not located on distinct tubercles; the lack of pupal spiracle sites; the weak chaetotaxy; the terminal posterior spiracular slit, which is fringed with conspicuous plumose hairs; and the streamlined, longitudinally striped body. Stratiomyine larvae are aquatic, whereas the cyphomyiines are scavengers in decaying plant materials; those of *C. **bicarinata***, from Arizona, were reared from decaying prickly pear, whereas those of *C.* sp. from Honduras were under the bark of cedar logs.

In many respects, the cyphomyiine larva bears a strong resemblance to the sargine and, to a more limited extent, to the hermetiine. These three subfamilies are much more similar to one another in head structure than any one of them is to the Stratiomyinae. Furthermore, they agree in the form and position of the prothoracic spiracle, in the dorsal or subterminal, unfringed posterior spiracular slit, and in the presence (though not in the detail) of the pupal spiracular horns. Also, the three longitudinal rows of bristles to each side of the median line of the thorax and abdomen are common to the three subfamilies, though the pattern in the Cyphomyiinae is distinctive. The ten-segmented, relatively unmodified antenna of the adult is a distinctive cyphomyiine character; but the Sarginae share with the Cyphomyiinae the primitive wing with its complete venation, the strong tendency toward metallic coloration, and the receding, or at least not produced, facial profile. The tendency toward a spined scutellum and toward expanded, often flared occipital orbits in the females, on the other hand, suggest the Stratiomyinae. The only logical position, on the basis of present evidence, is to consider *Cyphomyia* and its relatives as distinct from both the Sarginae and the Stratiomyinae, but closer to the former than to the latter.

ACKNOWLEDGMENTS

I am indebted to Dr. Clifford Berg for the loan of his drawings and for helpful suggestions, particularly for calling my attention to the pupal respiratory horns and their sites in the larva; to Dr. Willis E. Snow, for the loan of the material on which these descriptions were based; and to Mr. Paul Arnaud and the United States National Museum for the loan of the Honduras larvae and of other specimens which did not prove to be *Cyphomyia*.

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James, Maurice T. 1940. Studies in Neotropical Stratiomyidae (Diptera). IV. The genera related to *Cyphomyia* Wiedemann. Rev. de Ent., 11: 119–49.

BOOK NOTICE

A TEXTBOOK OF ENTOMOLOGY, second edition, by HERBERT H. ROSS. 8 vo., cloth, 519 pp., 402 figs., ref., index. New York, John Wiley & Sons, Inc. 1956. $7.75.

The second edition of this widely used text is a much improved revision of a book first published in 1948 (Review in Jour. Econ. Ent. 41: 996). Although the treatment and arrangement are identical and the total number of pages is slightly smaller, numerous corrections and additions as well as some deletions have been made. One of the significant improvements of the revised edition is the inclusion of many references to recent work at the end of each chapter and each order.

Dr. Ross's text is the only one published in the U.S.A. in which an attempt is made to present a balanced survey of the entire field of entomology for the beginning student in a limited number of pages which can be easily covered in a one-semester course. Although this is an admirable goal, it presents many inherent drawbacks. These were particularly evident in the pioneer first edition but have been somewhat alleviated in the revision. The greatest problem is that of adequate coverage of each subject without oversimplification and extreme generalization. The usual solution of this problem is to leave out all or most of the details, controversial points and conflicting views with the result that the student is left with the impression that either everything is obviously simple or that nothing is really known at all about the subject. It seems to be the general view among contemporary textbook writers that our college students lack the intelligence and maturity to grapple with details, are unable to draw their own conclusions (with some guidance, naturally) from the evidence presented, cannot understand anything but "basic" English, and that therefore they should be exposed only to the barest elemental material which will not tax their faculties nor burden them with details. There is little in such texts to stimulate a student, to point out avenues of needed and fruitful research, and to attract them to the field. A textbook should be more than just slightly polished lecture notes which a student can read without effort or memorize verbatim before an examination. Simplified texts, in my experience, are of little value to the instructor as well, for if he has a genuine interest in teaching he must present in his lectures much material which is not in the text and to which the student has no ready access, or is lacking the experience to evaluate.

Except for minor details of emphasis, the subjects covered in this textbook are essentially the same as those most teachers of general entomology have stressed in their courses for the last 20 years. Prior to the appearance of Dr. Ross' text several of these subjects had been neglected in American texts and were treated only in specialized and advanced books (Snodgrass, Wigglesworth, Johannsen and Butt) somewhat beyond the grasp of beginning students. In abstracting and interpreting the material from these sources for the original editor, Dr. Ross, in my opinion, attempted to simplify and condense it too much so that many of the generalizations presented had little or no evidence mentioned in their support and were therefore not convincing or even misleading. In the revised edition some of these generalizations have been eliminated, modified or qualified, but still require the student to take too much for granted and give him a false impression of the status of knowledge in the field. Despite these shortcomings the second edition of "A Textbook of Entomology" is a useful text for it contains the most up-to-date summaries in fields that are not covered in any other text. Undoubtedly it will be used even more widely than the first edition and will influence many students. Therefore I believe that the following detailed comments, derived from reading the book from cover to cover and from its actual use in my own course, should be presented in a spirit of constructive criticism.

In the first chapter on the "Growth of North American Entomology" (p. 1–25) little change has been made except for the inclusion of paragraphs on advances in economic entomology since 1944 and more up-to-date figures on financial losses due to insects.

The second chapter: "Arthropods: Insects and Their Allies" (p. 26–49) has been extensively reorganized, rewritten and condensed so that it is 8 pages shorter. Most of the condensation has been accomplished by the regrouping and elimination of figures and the exclusion of the bulk of the discussion of the Crustacea. The information presented is in general much less extensive than one would find in a general zoology text and is largely confined to superficial features. There is an unfortunate usage of the term biramous for the trilobite trunk appendages and the pauropod antenna. The figure of the pycnogonid has been inverted. Keys have been provided to the subclasses of Arachnoidea and some of the orders of Arachnida but none are available for the classes of the Arthropoda.

The principal revisions in the chapter on "External Anatomy" (p. 50–89) have been made in the sections on the origin of the head and on wing venation. The approach to the subject remains largely descriptive with very little attention paid to morphological principles and criteria. A greater amount of detail is presented in this chapter than anywhere else in the text. Despite this, no phase of insect morphology is treated in a manner that will enable a student to make interpretations or to determine homologies independently. It is unfortunate, in my opinion, that the mouthparts and the wing venation particularly are not covered more thoroughly. The morphology of immature stages is largely neglected. A few minor errors have been noted: only two ocelli are sometimes developed (p. 56); exite is used in the plural in fig. 43; pleurae is used on p. 71 while on the following page pleura is stated to be correct form; the pleural thoracic sclerites of apterygotes can be better described as rudimentary rather than degenerate (p. 73); although the pretarsus is described, it is not labelled in fig. 64.

The chapter on "Internal Anatomy" (p. 90–107) is extremely brief and has undergone very little revision. I cannot agree with the author that physiology should be treated separately from anatomy, for structure has little significance when it is divorced from function and vice versa. In the much more extensive chapter on "Physiology" (p. 108–162) much revision has been made, and many restrictions and qualifications of generalizations that were too broad. A new section has been added on the physiology of development which incorporates the former brief discussions of metamorphosis and diapause. There still remain numerous questionable statements, as for example: reference to the body wall or integument as an *organ* (p. 109); stretching of cuticle to accommodate *expected* size increase (p. 114); *pharynx* and *pharyngeal pump* for cibarium and cibarial pump (p. 117); no mention of histoblasts in connection with histogenesis (p. 143); no distinction between stemmata and ocelli (p. 153).

The "Life Cycle" chapter (p. 163–202) has undergone considerable revision in the text as well as in figures. The major changes are in the discussion of metamorphosis. It is unfortunate that such a fundamental and controversial subject as insect metamorphosis is treated only from one extreme point of view, without mention of any other interpretations. The use of a single superficial character as a criterion for the definition of complete from incomplete metamorphosis, with the resultant inclusion of such hemimetabolous forms as thrips and some Hemiptera in complete metamorphosis, is hardly justified even for the sake of simplicity. After all, convergence and parallelism are found in every phase

of insect biology and it is possible and necessary to recognize them. The section on aquatic, semi-aquatic and marine insects has been justifiably eliminated for there was little in this section that was not included under discussions of individual groups. Some errors remain undetected or have appeared in the revision, among them: insect eggs are better described as spheroids rather than *ellipses* (p. 164); the heart is derived from mesoderm not *ectoderm* (p. 170).

The chapter on "The Orders of Insects" (p. 203–403) forms, as in the first edition, roughly two-fifths of the volume. It has undergone considerable revision, addition, and some reorganization. There is a new, much needed introductory section on the evolution and phylogeny of insect orders, where an interpretation is presented that is particularly controversial in the Apterygota but no references are made to other interpretations. Portions of the key to orders, which is largely restricted to adult forms, have been revised. All the orders of primitively wingless insects have been lumped into the subclass Aptergygota. Many entomologists, including the reviewer, believe that there is ample justification for the recognition of separate subclasses for the Protura and Collembola, as was done in the first edition. On the other hand there seems to be little justification at this stage of our knowledge for the separation of the Microcoryphia as an order distinct from the Thysanura. The subclass Pterygota is now divided into the Paleoptera and the Neoptera and the latter into orthopteroid, hemipteroid and neuropteroid groups of orders. The discussions of the characteristics of these divisions are confined to the introductory section on evolution in which only one or two characters are mentioned. The Orthoptera *s. lat.* have been split into the Cursoria and Orthoptera; the Mallophaga, Anoplura and Rhynchophthirina have been united into the Phthiraptera; the Neuroptera *s. lat.* have been split into the Megaloptera, Neuroptera and Raphidiodea; the Strepsiptera remain as a suborder of the Coleoptera; the remaining orders are considered in the traditional fashion. The discussions of most of the orders are extremely elementary both with regard to morphology and ecology; those of the families are even more superficial. For example, for the characteristics of adult mosquitoes all we find is (p. 384): "Long-legged slender insects, fig. 329, having beadlike antennae (in the males they are plumose as in the Chironomidae) and many-veined wings. One small subfamily, the Chaoborinae (non-biting mosquitoes), have (*sic*) mouthparts that resemble pads. All the true mosquitoes belong to the subfamily Culicinae. In these the mouthparts form a beak, composed of a highly modified assemblage of piercing-sucking parts (see fig. 55, p. 70)." Although the excellent text and key illustrations often compensate somewhat for this brevity, this chapter is on the whole the weakest in the book. The keys to families, as in the first edition, are largely artificial and are restricted to the adults of the more common forms. Several new keys have been added (Collembola, Phthiraptera, Megaloptera, Neuroptera, Mecoptera, Siphonaptera) and others have been revised (Cursoria, Orthoptera, Apoidea, Diptera). I question the value of artificial keys in a textbook such as this or, for that matter, in any textbook. They properly belong in an identification manual, such as "An Introduction to the Study of Insects" by D. J. Borror and D. M. DeLong (New York, Rinehart, 1954). Artificial keys teach little except recognition characters. The characteristics of and variations in the various groups can be brought out in a textbook much more satisfactorily with phylogenetic keys. Among the new or uncorrected errors in this chapter, the following may be cited: in fig. 163 the Ephemeroptera are indicated as possessing a dicondylic mandible instead of the primitive monocondylic type (see also fig. 349); Machilidae is misspelled (p. 217); Locustidae is retained in preference to the more widely accepted Acrididae (p. 239); family names are often treated as being in the singular (p. 302 and elsewhere); Scarabaeidae is misspelled (p. 320, 337 but not in fig. 262, 263).

Chapter 8 on "Geological History of Insects" (p. 404–424) remains one of the outstanding features of the text. It has been very extensively revised, reorganized and amplified and it presents a much more coherent interpretation. It is evident that the author has reviewed this field recently in connection with his research. Perhaps here it is not necessary or even possible to go into greater detail or to mention divergent opinions. As mentioned earlier, fig. 349 does not agree with fig. 163 in regard to the type of mandibular articulation in the Ephemeroptera and the Thysanura.

The intimately related chapters on "Ecological Considerations" (p. 425–466) and "Control Considerations" (p. 467–499) have been considerably revised and partially brought up to date. A section on population dynamics has been added. In general most of the phases of the subjects discussed are illustrated by brief examples and some indication is made of variation. It is surprising that no discussion is presented of the beneficial effects of insects and also that mimicry, which is of such interest to beginning students, is dismissed in nine lines. Condensation and simplification give the wrong impression to the reader in several instances. For example, in dealing with the transmission of pathogens which undergo a developmental cycle within the internal environment of the insect, no mention is made of this essential cycle and one cannot help but draw the conclusion that these are merely special cases of mechanical transmission (p. 477). In the same section a number of organisms are mentioned under generic names which are no longer applicable: *Bacillus pestis*, *Filaria*, *Margaropus annulatus*.

Passing reference has been made to the simplified style used throughout the book. Certain portions, particularly discussions of families, are very loosely written and even give the impression of off-hand statements that have not been checked for either content or form. The illustrations are well chosen and in general of excellent quality. I like particularly the inclusion of numerous figures of immature stages. On the other hand, there is evident in the second edition a tendency toward oversimplification in illustrations. Some diagrams, which are already simplified and generalized representations, have had details or labels eliminated.

The attractive physical make-up of the first, letterpress, edition has been sacrificed to some extent in the second edition which is evidently reproduced by offset. All of the 15 copies of the book I have examined had numerous pages with a strongly washed-out appearance, particularly those with figures. Although the change in type faces and the reduction of the size of numerous figures has permitted slightly fuller pages, much of the saving of space has been lost in excessively large chapter headings and the rather frequent blank spaces adjoining small off-center figures. On the other hand, considerable improvement has been made in appearance and organization through the use of better center and side heads in a variety of typefaces including heavier boldface.

JOHN N. BELKIN,
University of California, Los Angeles

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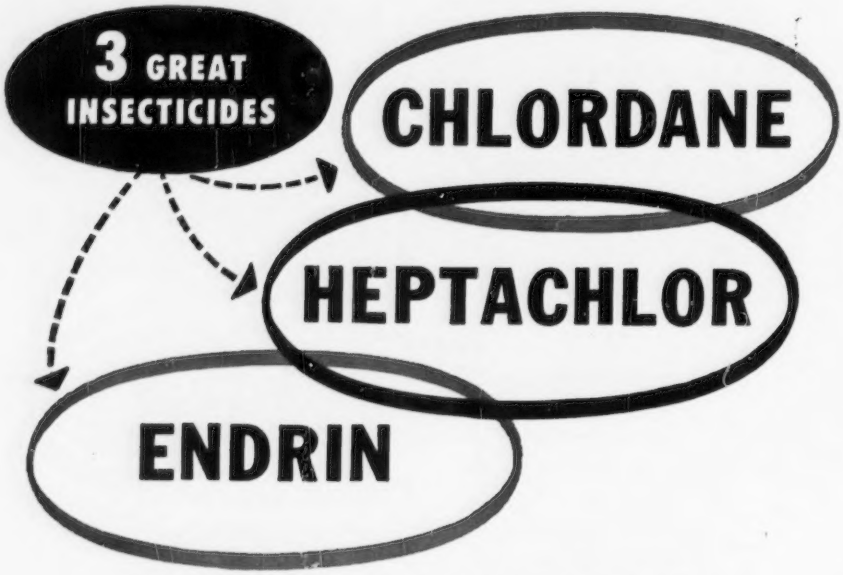

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TABLE OF CONTENTS

PAGE

TELFORD, ALLAN D.—The Pasture *Aedes* of Central and Northern California. The Egg Stage: Gross Embryology and Resistance to Desiccation 537

HOOGSTRAAL, HARRY—Bat Ticks of the Genus *Argas* (Ixodoidea, Argasidae). 2. *Secretargas* New Subgenus and *A. transgariepinus* White, 1846, Its Adult and Immature Stages; with a Definition of the Subgenus *Argus* 544

PHILIP, CORNELIUS B.—A New Era in Ideas of Systematic Relationships of World Tabanidae (Diptera) Inaugurated by Mackerras, and Its Impact on Nomenclature of Horseflies of the Western Hemisphere 550

HARWOOD, ROBERT F., AND WILLIAM R. HORSFALL—Development, Structure, and Function of Covering of Eggs of Floodwater Mosquitoes. I. Ovarian Development 555

AREEKUL, SUTHARM—The Comparative Internal Larval Anatomy of Several Genera of Scarabaeidae (Coleoptera) 562

DAVIS, G. R. F.—Growth and Feeding Behavior of Larvae of *Ctenicera aeripennis destructor* (Brown) (Coleoptera: Elateridae). I. Effects of Carrot Slices and Seeds of Wheat, Flax, Barley, Rye, and Alfalfa 578

FROST, S. W., AND J. O. PEPPER—Aphids Attracted to Light Traps 581

ALEXANDER, RICHARD D.—The Taxonomy of the Field Crickets of the Eastern United States (Orthoptera: Gryllidae: *Acheta*) 584

LEWALLEN, LAWRENCE L.—Paper Chromatography Studies of the *Anopheles maculipennis* Complex in California (Diptera: Culicidae) 602

FENTON, F. A., AND D. E. HOWELL—A Comparison of Five Methods of Sampling Alfalfa Fields for Arthropod Populations 606

DARSIE, RICHARD F., JR.—Notes on American Mosquito Pupae II. The *Aedes* (*Ochlerotatus*) *punctor* Subgroup, with a Key to Known Nearctic *Aedes* Pupae (Diptera, Culicidae) 611

MEDLER, J. T., AND T. W. KOERBER—Biology of *Dipogon sayi* Banks (Hymenoptera, Pompilidae) in Trap-nests in Wisconsin 621

WALKER, THOMAS J., JR.—Specificity in the Response of Female Tree Crickets (Orthoptera, Gryllidae, Oecanthinae) to Calling Songs of the Males 626

EBELING, WALTER, AND ROY J. PENCE—The Use of Pesticides in the Preparation of Insects and Mites for Photography 637

JAMES, MAURICE T.—The Larva of *Cyphomyia* (Diptera, Stratiomyidae) and Its Significance in Classification 639

Book Notices 543, 642

INDEX TO VOLUME 50 644